

**CHANGES IN THROMBIN
ACTIVATABLE FIBRINOLYSIS
INHIBITOR AND ITS ROLE IN
NORMAL PREGNANCY AND
PRE-ECLAMPSIA**

BY

FARAH YASMINE HUQ BMedSci BMBS MRCS

A thesis submitted in the fulfilment for the degree of Doctor of Medicine (MD)

University College London

April 2017

DECLARATION:

I declare that the work presented in this thesis is my own and has not been submitted to another university.

Signature:

Date: 1st April 2017

Name: Farah Yasmine Huq

ACKNOWLEDGEMENTS:

I would like to offer my deepest thanks to my supervisors Miss Rezan Abdul-Kadir and Professor Edward Tuddenham for their unerring patience, wisdom and kindness.

This thesis would not have been possible without the patients who generously gave up their time to participate in these studies, I would like to express my gratitude and wish the all the best with their new babies and their future endeavours.

My sincere gratitude must also go to the staff of the Haemophilia Centre at the Royal Free Hospital, especially Anne Riddell. Equally, many thanks to Mr Whitlow and his team at Colchester hospital for all of their help.

Finally, for Matthew and Oscar.

TABLE OF CONTENT

TITLE

DECLARATION

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

Chapter 1: Introduction to MD thesis

- 1.1 Introduction
- 1.2 Study aims and objectives
- 1.3 Thesis layout

Chapter 2: Literature Review

- 2.0 Overview of Haemostasis
- 2.1 Coagulation
 - 2.1.1 Extrinsic Pathway
 - 2.1.2 Intrinsic Pathway
 - 2.1.3 Final Common Pathway
 - 2.1.4 Regulation of coagulation
- 2.2 Fibrinolysis
- 2.3 Haemostasis in normal pregnancy
 - 2.3.1 Changes in procoagulants
 - 2.3.2 Changes in anticoagulants
 - 2.3.3 Fibrinolysis
 - 2.3.4 Thrombin Generation
 - 2.3.5 Placental Haemostasis
 - 2.3.6 Platelets
- 2.4 Thrombin activatable fibrinolysis inhibitor
 - 2.4.1 Discovery
 - 2.4.2 Synthesis of TAFI
 - 2.4.3 TAFI activation
 - 2.4.4 Physiological roles of TAFI
 - 2.4.4.1 The attenuation of fibrinolysis
 - 2.4.4.2 The role of TAFI in inflammation
 - 2.4.5 Instability of TAFI
 - 2.4.6 TAFI inhibitors
 - 2.4.7 Pathophysiology of TAFI
 - 2.4.8 TAFI in Pregnancy
 - 2.4.8.1 TAFI in normal pregnancy
 - 2.4.8.2 TAFI in pre eclampsia
 - 2.4.8.3 TAFI in recurrent miscarriage and implantation failure
- 2.5 Pre eclampsia
 - 2.5.1 Definition
 - 2.5.1.a Definition of severe pre eclampsia
 - 2.5.1.b Early and late pre eclampsia
 - 2.5.2 Maternal morbidity and mortality
 - 2.5.3 Risk Factors
 - 2.5.3.a Parity and parternity
 - 2.5.3.b Maternal age

- 2.5.3.c Ethnicity
- 2.5.3.d Medical co-morbidities
- 2.5.3.e Increased placental mass
- 2.5.3.f Obesity
- 2.5.3.g Assisted conception
- 2.5.4 Cardiovascular risk after pre eclampsia
- 2.5.5 Clinical features
 - 2.5.5.a Signs and symptoms
- 2.5.6 Pathogenesis of pre eclampsia
 - 2.5.6.a Role of the placenta
 - 2.5.6.b Vascular development of the placenta
 - 2.5.6.c Altered angiogenic balance
 - 2.5.6.d Placental ischaemia and hypoxia
 - 2.5.6.e Maternal endothelial dysfunction
 - 2.5.6.f Haemodynamic changes
 - 2.5.6.g Immune changes
 - 2.5.6.h Hepatic, renal and cerebral changes
- 2.5.7 Current screening methods for pre eclampsia
 - 2.5.7.a Maternal history
 - 2.5.7.b Blood pressure
 - 2.5.7.c Uterine artery dopplers
 - 2.5.7.d Biomarkers
- 2.5.8 Prevention of pre eclampsia
- 2.5.9 Haemostasis in pre eclampsia
 - 2.5.9.a Haemostasis in the systemic circulation
 - 2.5.9.b Fibrinolysis in pre eclampsia
 - 2.5.9.c Uteroplacental circulation

Chapter 3: Laboratory Method

- 3.0 TAFI antigen and activity assays
- 3.1 Immunological assays
- 3.2 Enzymological assays
- 3.3 Functional assays
- 3.4 Laboratory method for the studies in this thesis
 - 3.4.1 TAFI Activity (TAFI Actichrome[®])
 - 3.4.2 TAFI Antigen concentration (ELISA)
 - 3.4.3 Clot Lysis time

Chapter 4: Changes in Thrombin activatable fibrinolysis inhibitor in normal pregnancy

- 4.1 Introduction
- 4.2 Method
 - 4.2.1 Study design
 - 4.2.2 Sample size
 - 4.2.3 Inclusion and exclusion criteria
 - 4.2.4 Data collection
 - 4.2.5 Blood collection
 - 4.2.6 Laboratory method
 - 4.2.7 Statistics

- 4.3 Results
 - 4.3.1 Population and demographic data
 - 4.3.2 Changes in TAFI Ag, TAFIa and CLT with gestation
 - 4.3.3 Reference ranges for TAFI in normal pregnancy
 - 4.3.4 CLT in the presence of Buffer and PTCI
- 4.4 Discussion

Chapter 5: Thrombin activatable fibrinolysis inhibitor in pre eclampsia

- 5.1 Introduction
- 5.2 Method
 - 5.2.1 Study design
 - 5.2.2 Inclusion and exclusion criteria
 - 5.2.3 Data collection
 - 5.2.4 Blood collection
 - 5.2.5 Laboratory method
 - 5.2.6 Statistical analysis
- 5.3 Results
 - 5.3.1 Demographic data
 - 5.3.2 Changes in mean levels of TAFI Ag, TAFIa and CLT in pre eclampsia
 - 5.3.3 Comparison of TAFI in normal pregnancy and pre eclampsia
 - 5.3.4 CLT in the presence of buffer and PTCI
 - 5.3.5 Correlation between TAFI Ag, TAFIa and CLT in pre eclampsia
- 5.4 Discussion

Chapter 6: Overall conclusions

- 6.1 Overall conclusions
- 6.2 Suggestions for future research

Appendix 1: Study Ethical approval

REFERENCES

LIST OF TABLES AND FIGURES:

TABLES:

1. Studies of changes in TAFI Ag and TAFIa in normal pregnancy and Pre-eclampsia
2. American College of Obstetrics and Gynecology Criteria for severe pre eclampsia
3. Medical conditions associated with pre eclampsia
4. Materials required for TAFI antigen and activity levels and Clot lysis time analysis
5. Number of samples analysed in each trimester of normal pregnancy
6. Distribution of age, parity, booking weight and blood pressure in normal pregnancy
7. a) Changes in TAFI Ag, TAFIa and CLT throughout normal pregnancy
b) Statistical significance of changes in the mean values of TAFI Ag, TAFIa and CLT in normal pregnancy
8. Reference intervals of TAFI Ag and TAFIa during each trimester of pregnancy
9. Reference ranges of TAFI Ag and TAFIa in normal pregnancy
10. Contribution of TAFI to fibrinolysis assessed by the the difference between CLT in the presence of buffer and PTCI
11. Correlation of TAFI Ag and TAFIa with CLT in each trimester of pregnancy
12. Number of women with pre eclampsia recruited in each trimester
13. Distribution of age, parity, booking weight and blood pressure in pre eclampsia
14. a) Changes in mean TAFI Ag, TAFIa and CLT throughout pregnancy complicated by PET
b) Statistical significance of changes in the mean values of TAFI Ag, TAFIa and CLT in pre eclampsia
15. Comparison of mean TAFI Ag, TAFIa and CLT in normal pregnancy and pre eclampsia
16. Contribution of TAFI to fibrinolysis assessed by the the difference between CLT in the presence of buffer and PTCI in pre eclampsia
17. Correlation between TAFI Ag and TAFIa with CLT in pre eclampsia

FIGURES:

1. The coagulation cascade
2. The fibrinolytic pathway
3. The role of TAFI in fibrinolysis
4. Changes in mean TAFI Ag levels throughout normal pregnancy
5. Changes in mean TAFIa levels throughout normal pregnancy
6. Changes in mean CLT throughout normal pregnancy
7. Changes in TAFI Ag levels with gestation
8. Changes in TAFIa levels with gestation
9. Distribution of TAFIa in each trimester of normal pregnancy
10. Distribution of TAFI Ag in each trimester of normal pregnancy
11. Distribution of CLT in each trimester of normal pregnancy
12. The distribution of TAFa in the first trimester
13. The distribution of TAFa in the second trimester
14. The distribution of TAFa in the third trimester
15. The distribution of TAFI Ag in the first trimester
16. The distribution of TAFI Ag in the second trimester
17. The distribution of TAFI Ag in the third trimester
18. a) Correlation between TAFI Ag and CLT in the first trimester
b) Correlation between TAFIa and CLT in the first trimester
19. a) Correlation between TAFI Ag and CLT in the second trimester
b) Correlation between TAFIa and CLT in the second trimester
20. a) Correlation between TAFI Ag and CLT in the third trimester
b) Correlation between TAFIa and CLT in the third trimester
21. The distribution of TAFI Ag in the second and third trimesters in PET
22. The distribution of TAFIa in the second and third trimesters in PET
23. The distribution of CLT in the second and third trimesters in PET
24. a) Mean TAFI Ag levels throughout pregnancy complicated by PET
b) Mean TAFIa levels throughout pregnancy complicated by PET
c) Mean CLT throughout pregnancy complicated by PET
25. Correlation between TAFI Ag and CLT in the third trimester in pre-eclampsia
26. Correlation between TAFIa and CLT in the third trimester in pre-eclampsia

Chapter 1

INTRODUCTION TO MD THESIS

The Activity of Thrombin Activatable Fibrinolysis Inhibitor (TAFI) in pregnancy affected by Pre-Eclampsia

1.1 Introduction

Thrombin activatable fibrinolysis inhibitor (TAFI) is a recently described glycoprotein that inhibits fibrinolysis (Bajzar, Manuel et al. 1995). It can be converted to its active form, TAFIa, by proteolytic cleavage by the thrombin/thrombomodulin complex. TAFIa inhibits fibrinolysis by removing carboxyterminal residues from partially degraded fibrin and decreasing plasminogen binding on the surface of fibrin (Bajzar, Manuel et al. 1995; Redlitz, Tan et al. 1995; Bajzar, Morser et al. 1996). TAFI appears to play an important part in the delicate balance between coagulation and fibrinolysis, but its role in pathology requires further elucidation. It seems likely that the activity of TAFI is altered in various thrombotic and bleeding disorders. Increased levels of TAFI antigen have been reported in patients with symptomatic coronary artery disease (Silveira, Schatteman et al. 2000) and as a risk factor for deep vein thrombosis (van Tilburg, Rosendaal et al. 2000). Decreased TAFI activity has been observed in acute promyelocytic leukaemia (Meijers, Oudijk et al. 2000) and disseminated intravascular coagulation (Watanabe, Wada et al. 2001).

In normal pregnancy there are increased levels of procoagulation factors, enhanced thrombin generation and impaired fibrinolysis (Bremme, Ostlund et al. 1992; Comeglio, Fedi et al. 1996). Since thrombin generation is increased during pregnancy, TAFI may contribute to the decreased fibrinolytic state.

Pre-eclampsia is a complication of pregnancy induced by impaired placental circulation and is associated with haemostatic disturbances commonly observed as the activation of coagulation and the impairment of fibrinolysis (Perry and Martin 1992; Gilabert, Estelles et al. 1995; Schjetlein, Haugen et al. 1997; Sheppard and Bonnar 1999). This leads to the hypothesis that TAFI is involved in the regulation of fibrinolysis in women with these complications of pregnancy.

Several investigators have studied TAFI antigen levels during pregnancy (Chetaille, Alessi et al. 2000; Chabloz, Reber et al. 2001; Antovic, Rafik Hamad et al. 2002; Alacacioglu, Ozcan et al. 2004; Mousa, Downey et al. 2004) and produced conflicting findings. Chetaille et al found no significant difference in antigen levels between pregnant women and age matched controls, although the study was limited by a small sample size (n=12) (Chetaille, Alessi et al. 2000). Subsequent larger studies found that TAFI antigen levels increased with gestation (Chabloz, Reber et al. 2001; Mousa, Downey et al. 2004). Two previous studies have investigated the TAFI antigen levels in pre-eclampsia (Antovic, Rafik Hamad et al. 2002; Alacacioglu, Ozcan et al. 2004), with one including pregnancies affected by intrauterine growth retardation (IUGR) (Alacacioglu, Ozcan et al. 2004). The two studies produced conflicting results: Antovic et al (Antovic, Rafik Hamad et al. 2002) found lower levels of TAFI antigen in pre-eclamptic women, whereas Alacacioglu et al (Alacacioglu, Ozcan et al. 2004) did not find a statistically significant difference. None of the above studies have examined the levels of activated TAFI (TAFIa). Watanabe et al is the only study in the literature that looked at the changes in the concentration of plasma TAFI molecules that are present and functional after activation by thrombin-thrombomodulin complex

(TAFI activity) during pregnancy (Watanabe, Minakami et al. 2004). They reported higher TAFI activity in pregnant women when compared with non-pregnant women.

The following thesis aims to investigate if and how TAFI levels change during normal pregnancy. This will provide a reference range for TAFI in uncomplicated pregnancy, allowing comparison of the changes in the activity of TAFI in plasma during pregnancies complicated by pre-eclampsia or IUGR contributes to a better understanding of the pathophysiology of these conditions. TAFI may have a future role in the screening and monitoring of these conditions.

1.2 Study Objectives and Aims:

- **To determine the nature of changes in TAFI antigen and activity levels during normal pregnancy;**
- **To determine pregnancy specific reference intervals for TAFI Ag and TAFIa**
- **To determine if TAFI antigen or TAFI activity levels correlate with clot lysis time;**
- **To determine if women with pre-eclampsia have altered TAFI antigen and activity levels compared with normal pregnant controls and if so, does this contribute to impaired fibrinolysis**

1.3 Thesis layout

The second chapter of the thesis begins with a brief overview of haemostasis and how it is affected in normal pregnancy. The latter parts of chapter 2 provide a background about TAFI, including its role in haemostasis and the pathophysiology of various disease states. The final section deals with the current understanding of the pathophysiology of PET and changes in haemostasis in the disease.

The third chapter provides details of the current methods available to assess TAFI levels and its effects. The laboratory methods used in the studies forming the basis of this thesis are elucidated in this chapter.

Chapter 4 details a study of changes in TAFI in normal pregnancy and chapter 5 compares TAFI levels in PET to the reference range for normal pregnancy defined in chapter 4.

The last chapter of the thesis summarises the findings of the thesis and explains their importance with suggestions for further research.

Chapter 2

LITERATURE REVIEW

2.1: An overview of normal haemostasis

A brief revision of the coagulation cascade is essential to understand the haemostatic changes in pregnancy. The cascade is a three-part system (constituting of the intrinsic, extrinsic and final common pathway) of interlinked positive feedback loops that act to form a stable blood clot. Blood remains fluid because: (i) coagulation proteins circulate in their inactive form; (ii) unlike the sub-endothelium the endothelium is devoid of thrombogenic Tissue Factor (TF) or collagen and finally (iii) the brisk velocity of flow removes any activated proteins to be metabolised in the liver.

2.1 Coagulation

2.1.1 Extrinsic Pathway:

The primary stimulus for the initiation phase of the extrinsic pathway is the exposure of the sub-endothelium subsequent to vessel damage. This causes the release of tissue factor (TF), which then associates with FVIIa to form a complex of FVIIa-TF. Together with calcium and the negatively charged phospholipid surface, FVIIa-TF forms the extrinsic FXase.

2.1.2 Intrinsic Pathway:

Exposure of the sub-endothelial surface stimulates circulating factor XIIa to cleave and activate its substrates Prekallekrein and Factor XI into Kallekrein and Factor XIa. Positive feedback occurs as Kallekrein activates neighbouring FXII molecules. Kallekrein and FXIa become anchored to the sub-endothelium by kininogen. Factor XIa's serine protease activity cleaves the anchoring kininogen, making FXIa soluble enabling it to activate FIX into FIXa.

Simultaneously, the small amount of thrombin generated by the extrinsic pathway promotes the activation of platelets and factors V, VIII, and XI into FVa, FVIIIa and FXIa respectively (Lawson, Kalafatis et al. 1994; Butenas, van 't Veer et al. 1997). In a similar manner to FVII, FVIII forms the intrinsic FXase together with IXa, calcium and the phospholipid surface. The intrinsic pathway generates a much larger amount of thrombin compared to the extrinsic pathway, due to its FXase being 50-100 fold more active (Lawson and Mann 1991; Ahmad, Rawala-Sheikh et al. 1992). This is the final step of the intrinsic pathway and leads to the subsequent final common pathway.

2.1.3 Final Common Pathway:

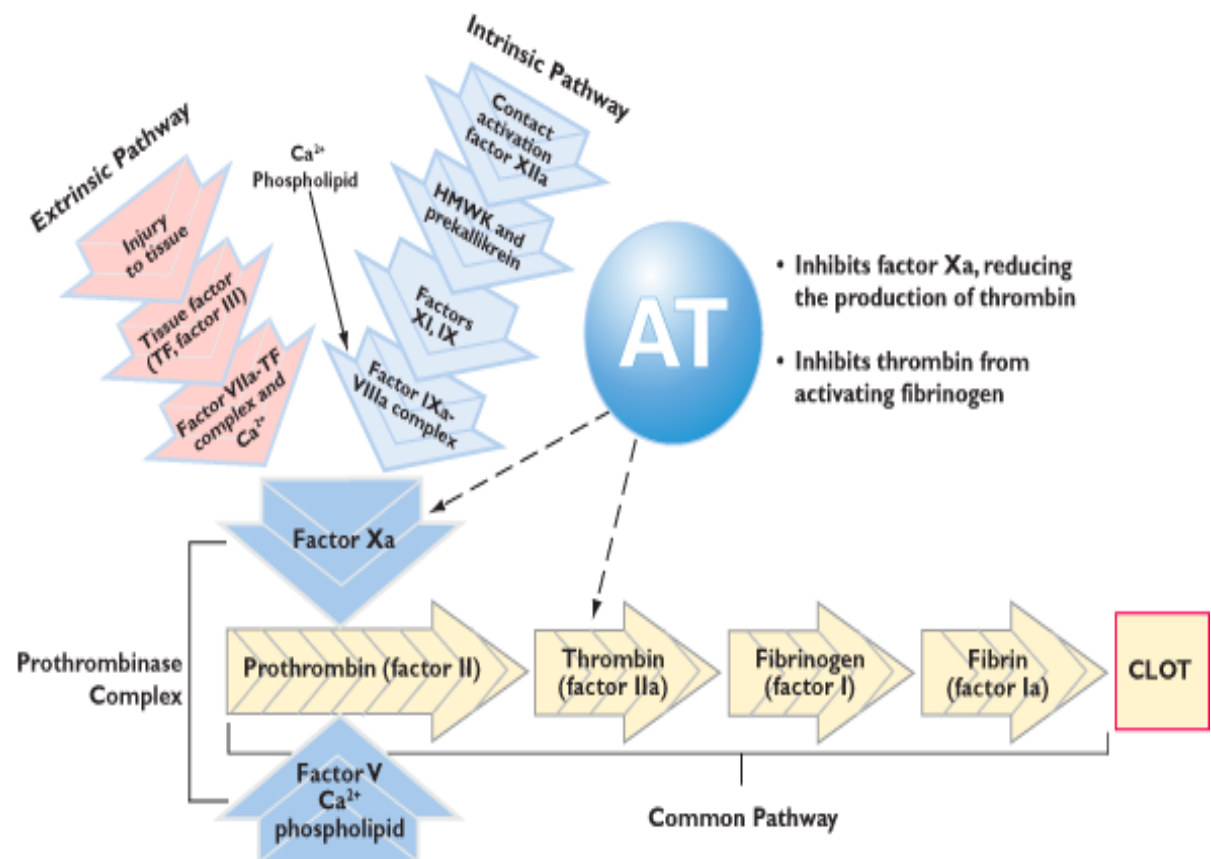
When activated, FXa associates with its cofactor FVa in the presence of calcium and the phospholipid surface, to form a prothrombinase complex. This complex subsequently converts prothrombin into thrombin. By complexing with FVa, the rate of FXa mediated thrombin generation increases by 10^5 fold (Pryzdial and Mann 1991). Thrombin is the terminal serine protease of the coagulation cascade and its main action is to cleave fibrinogen to expose polymerisation sites. These sites allow each fibrin molecule to form non-covalent bonds with two neighbouring fibrin molecules, forming double stranded protofibrils, which then arrange themselves into the three-dimensional network of the stable fibrin clot. The structure of the polymerised fibrin is stabilised further by FXIIIa (which is activated by thrombin). FXIIIa forms covalent bonds between fibrin molecules within each protofibril (Hornyak and Shafer 1992; Weisel, Francis et al. 1993).

2.1.4 Regulators of coagulation

Tissue factor pathway inhibitor (TFPI) acts to inhibit the actions of the extrinsic FXase, in addition to FX (Broze, Warren et al. 1988; Rapaport 1991). Thrombomodulin (TM) is expressed throughout the vascular endothelium (Esmon 1995) and binds with thrombin forming the IIa-TM complex, which in turn activates protein C (APC). APC is an anti-coagulant that inhibits FV and FVIII activation.

Antithrombin (AT), is present in low plasma concentrations and thus inactivates thrombin at a relatively slow rate. In addition to thrombin, AT has an inhibitory effect on FIXa, FXa, FXIa and FXIIa and plasmin.

Figure I: The Coagulation cascade (Escobar, Harmening et al. 2002)



Adapted from Escobar et al.¹

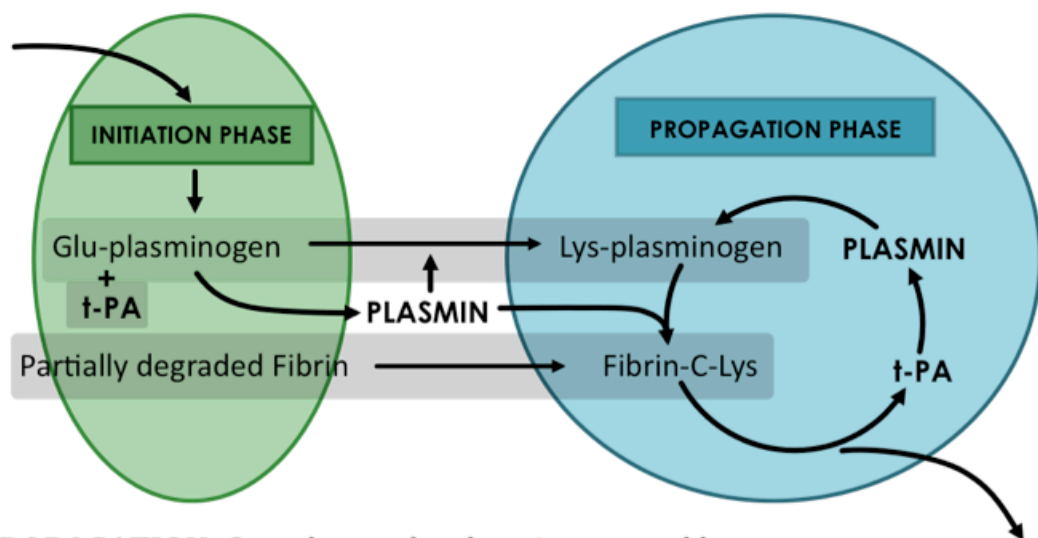
2.2 Fibrinolysis

The fibrinolytic system removes thrombus that is formed during the repair of the vascular endothelium, restoring patency to the vessel. However, the fibrinolytic system also has roles in tissue remodelling, tumour invasion, ovulation and in the implantation and early development of the fetus (Loskutoff 1988). Plasmin is the central component of the fibrinolytic system and acts to lyse fibrin clots. Plasmin is formed by the activation of its pro-enzyme plasminogen by fibrinolytic activators including tissue type plasminogen activator (tPA), which is released by the damaged vessel wall, and to a lesser extent, urokinase type plasminogen activator (uPA) which is present in low plasma concentrations. Plasminogen activator inhibitors (PAI 1 produced by the endothelium and PAI 2 produced by the placenta) regulate the activity of tPA and uPA. PAI I acts upon cell surface receptors and matrix binding points, so its proteolytic actions are specifically localised. This is important in the regulation of the fibrinolytic system (Rijken, Otter et al. 1990).

Intravascular fibrinolysis is initiated when plasmin and tPA bind to lysines on the surface of fibrin (this is known as the initiation phase of fibrinolysis). Plasmin partially degrades fibrin, exposing C-terminal lysine residues which have several positive feedback type actions: i) the C-terminal lysines upregulate plasmin generation by increasing plasminogen's affinity for partially degraded fibrin and fibrin degradation products (FDP); ii) the conversion of Glu¹-plasminogen to Lys⁷⁸-plasminogen by plasmin, makes plasminogen a better substrate for tPA; and finally iii) the C-terminal lysine residues decrease the rate of plasmin inhibition by α_2 -antiplasmin, which is unable to act upon plasmin bound to fibrin or FDP. All of these actions greatly increase the efficacy of fibrinolysis, and these positive feedback loops are termed the acceleration phase of fibrinolysis.

Figure 2: The fibrinolytic pathway

- INITIATION:** Fibrin clot partially degraded by plasmin



- PROPAGATION:** Complexes glu-plasminogen and lys-plasminogen act as substrates for further formation of plasmin
- Plasmin truncates glu-plasminogen to lys-plasminogen
- Cyclic: plasmin formation and fibrinolysis

2.3 Haemostasis in normal pregnancy

Normal pregnancy is associated with changes in both the coagulation and fibrinolytic systems. The concentrations of the majority of coagulation factors increase; levels of some physiological anticoagulants decrease and components of the fibrinolytic system are reduced (Bremme 2003; Brenner 2004). Overall, this creates a hypercoaguable state which is most marked towards term and persists through the post-partum period. Although these adaptations may be an important factor to limit blood loss during delivery and maintain placental function, they also increase the risk of venous thromboembolism during pregnancy and the puerperium.

2.3.1 Changes in procoagulants:

There is a progressive, significant rise in the levels of most coagulation factors including factor VII, VIII, X, XII, von Willebrand factor (VWF) and plasma fibrinogen (Stirling, Woolf et al. 1984; Aharon, Lanir et al. 2005; Kadir R 2009). The increase in coagulation factor levels is generally most marked in the third trimester, possibly in preparation for delivery (Hellgren and Blomback 1981; Stirling, Woolf et al. 1984; Clark, Brennand et al. 1998; Kadir RA 1998; Kadir R 2009). Factors II, V and IX have all been reported to be either slightly increased or unchanged during pregnancy (Nilsson and Kullander 1967; Stirling, Woolf et al. 1984).

Plasma fibrinogen concentration in late pregnancy and labour is up to two fold non pregnant levels (Stirling, Woolf et al. 1984) and FVII can increase by up to ten fold. FVIII and VWF concentrations progressively increase from as early as six weeks gestation to peak at term and then return to pre pregnancy levels by six weeks post partum (Stirling, Woolf et al. 1984; Sanchez-Luceros, Meschengieser et al. 2003). Factor XI decreases slightly, reaching concentrations of 60-70% of the normal non

pregnant value and reaches a nadir at term (Hellgren and Blomback 1981). Factor XIII (subunit A antigen) initially increases by 40% by mid pregnancy but then levels fall, reaching non pregnant levels by term (Persson, Stenberg et al. 1980). Factors II and V remain unchanged during pregnancy (Stirling, Woolf et al. 1984).

2.3.2 Changes in anticoagulants:

Levels of TM, which when bound to thrombin activates Protein C, are decreased after 32 weeks gestation (Boffa, Valsecchi et al. 1998). This is due to neutrophil activation which triggers proteolysis of endothelial TM, releasing soluble TM into the circulation. Despite TM levels being uniform amongst pregnancies until 12 weeks, the wide range reported after this gestation makes predicting preeclampsia or gestational hypertension using a TM reference curve unreliable (Boffa, Valsecchi et al. 1998). However, a sudden increase from a baseline value in an individual can indicate an underlying placental vascular disorder.

Protein C activity may fluctuate slightly during pregnancy but all values remain within the normal non-pregnant range (Gonzalez, Alberca et al. 1985; Aznar, Gilabert et al. 1986; Gilabert, Fernandez et al. 1988; Bremme, Ostlund et al. 1992; Faught, Garner et al. 1995; Kjellberg, Andersson et al. 1999). Protein S exists either as functionally active free Protein S, or as an inactive complex bound to C4-B binding protein. C4-B binding protein levels increase during pregnancy, but this accompanied by a fall in free protein S in the second and third trimesters (Gilabert, Fernandez et al. 1988; Brenner 2004). It remains unknown if the fall in free protein S contributes to the hypercoagulable state of pregnancy. Overall, there is a progressive decrease of total protein S with increasing gestation (Comp, Thurnau et al. 1986; Lefkowitz, Clarke et al. 1996).

2.3.3 Fibrinolysis:

At present, there is no consensus opinion on the changes in fibrinolytic activity during pregnancy. Several studies report that fibrinolysis is increased during pregnancy (van Wersch and Ubachs 1991; Cadroy, Grandjean et al. 1993; Cerneca, Ricci et al. 1997; Uchikova and Ledjev 2005), whereas others report that it is suppressed, (Stirling, Woolf et al. 1984; Holmes, Wallace et al. 2002; Bremme 2003; Clark 2003) with normal levels being restored within 1 hour of delivery of the placenta (Stirling, Woolf et al. 1984). Furthermore, some authors are of the opinion that none of the observed changes in the components of the fibrinolytic system have an effect on overall fibrinolytic activity (Kruithof, Tran-Thang et al. 1987; Wright, Cooper et al. 1988).

Previous reports of increased concentrations of fibrin degradation products (FDP) in pregnancy had led former authors to conclude that fibrinolysis activity was increased during pregnancy (Uchikova and Ledjev 2005). However, it has been suggested that increased levels of FDP such as D dimmers originate from the increased fibrin generation and degradation within the utero-placental unit (plasma D dimmer levels are higher in women who have a Caesarean section during labour compared to those who have an elective procedure), despite a reduced fibrinolytic potential in the systemic circulation (Bremme 2003). A further possible explanation for increased levels of circulating FDP is impaired clearance (Wright, Cooper et al. 1988).

Increased levels of plasminogen inhibitors PAI 1 and 2 act to decrease levels of tissue plasminogen activator (tPA) (Bremme 2003; Brenner 2004). PAI 2 is produced by placental villous cells and is undetectable outside of pregnancy (Astedt, Hagerstrand et al. 1986). There is a positive correlation between placental mass and PAI 2 levels (de

Boer, ten Cate et al. 1989). Furthermore, PAI 2 levels also vary with fetal size, suggesting that levels are dependant upon the quality as well as the quantity of placental tissue (He, Bremme et al. 1996).

2.3.4 Thrombin generation:

Activation of the coagulation cascade leads to the formation of thrombin. Thrombin is a serine protease which has several actions including the formation of fibrin and the activation of platelets and factors V, VIII and XIII. Thrombin generation starts to increase from as early as 7 weeks gestation to peak at term (Rosenkranz, Hiden et al. 2008). Thrombin generation can be assessed by quantifying circulating levels of marker proteins formed during thrombin generation or inactivation. These include soluble fibrin, thrombin-antithrombin complexes (TAT) and prothrombin fragments 1 and 2 (F1 and 2). In a study of 26 normal pregnant women, 50% had increased TAT levels in the first trimester and all of the participants had significantly increased levels during the second and third trimesters (Bremme, Ostlund et al. 1992). In addition, soluble fibrin was seen to increase progressively from the first trimester until term (Bremme, Ostlund et al. 1992). F1 and 2 have been demonstrated to be elevated beyond non-pregnant levels from the first trimester and there is a positive correlation between gestation age and increased levels (Comeglio, Fedi et al. 1996). Elevated antenatal levels of soluble fibrin TAT and F1 and 2, suggest that increased thrombin generation is a feature of normal pregnancy (de Boer, ten Cate et al. 1989).

2.3.5 Placental Haemostasis:

In addition to changes in systemic haemostasis, there is evidence of marked changes within the utero-placenta circulation. Maternal blood flows through the decidual arteries into the intervillous space and henceforth into the syncytiotrophoblast cells. In order to accommodate increasing maternal blood flow into the intervillous space, there is a physiological adaptation of the spiral arteries. Much of their vascular endothelium and underlying smooth muscle are replaced by trophoblasts. For the placenta to function efficiently, there must be a balance of rapid activation and close local regulation of coagulation (Lanir, Aharon et al. 2003). As gestation increases, the syncytiotrophoblast starts to express coagulation components including VWF and the CD31 marker in addition to tissue factor (TF) (Kuczynski, Uszynski et al. 2002). TF, the primary cellular mediator of haemostasis, is also expressed on perivascular decidualized human endometrial stromal cells (HESC), in order to prevent haemorrhage as a result of trophoblastic invasion (Beller and Ebert 1982). In addition to their haemostatic actions, coagulation components including TF and TM have a role in placental blood vessel differentiation (Lockwood, Krikun et al. 2000).

Placental fibrin deposits are seen in normal pregnancy (Clark and Greer 2003). This suggests that the decrease in fibrinolysis seen in plasma is a mechanism to inhibit fibrin degradation in the placenta to prevent haemorrhage during pregnancy and delivery. Furthermore, when compared with endothelial vasculature, trophoblasts exhibit a reduced capacity to lyse fibrin due to high levels of PAI 1 and 2 (Sheppard and Bonnar 1999).

Compared to maternal plasma, amniotic fluid contains higher levels of TF (Erez, Gotsch et al. 2009). Subsequently, amniotic fluid has procoagulant activity, with the ability to accelerate clot formation in in vitro testing (Weiner, Reid et al. 1949).

2.3.6 Platelets:

Thrombocytopenia (a platelet count < 150) is the most common haemostatic abnormality found antenatally and affects 7.3-11.6% of normal term pregnancies (Bremme 2003). Antenatal thrombocytopenia does not warrant further investigation unless it is detected in early pregnancy, the patient is symptomatic or the platelet count is less than 100. Platelet count progressively decreases with gestation, falling significantly between 12 and 24 weeks (Bremme, Ostlund et al. 1992). There is an associated decrease in mean platelet volume (Fay, Hughes et al. 1983). This implies that there is increased platelet production to compensate for enhanced platelet destruction as a result of low-grade intravascular coagulation within the placenta (Fay, Hughes et al. 1983). Platelet activation and aggregation are also enhanced during pregnancy leading to increased plasma concentrations of CD63 (Holthe, Staff et al. 2004) and β -thromboglobulin (Douglas, Shah et al. 1982).

Pregnancy is associated with increased activation of the maternal coagulation cascade resulting in increased thrombin generation. This may be a consequence of a combination of factors including altered hormone-influenced synthesis, an increased volume of distribution and increased catabolism. This hypercoagulable state is a physiological response to the haemostatic challenges of pregnancy. However, abnormalities of the thrombotic/ antithrombotic balance in early pregnancy may act as markers for subsequent vascular disorders including pre-eclampsia.

2.4 Thrombin activatable fibrinolysis inhibitor Protein:

2.4.1 Discovery:

In 1989, two independent studies identified the proteins with carboxypeptidase activity distinct from the constitutively active plasma carboxypeptidase N (Campbell and Okada 1989; Hendriks, Scharpe et al. 1989). The proteins were named unstable carboxypeptidase (CPU) and arginine carboxypeptidase (CPR). In 1991, Eaton et al isolated a plasminogen binding protein (plasma pro CPB) with an amino acid sequence similar to pancreatic carboxypeptidase B (Eaton, Malloy et al. 1991), and in 1995 Bajzar et al characterized a carboxypeptidase which, once activated by thrombin, attenuated clot lysis (Bajzar, Manuel et al. 1995). The presence of these proteins in plasma can explain the anti-fibrinolytic effect of the activation of prothrombin during t-PA activated fibrinolysis. Eventually, amino acid terminal sequencing revealed that all four of the proteins identified (CPU, CPR, plasma pro CPB and TAFI) were identical (Vanhoof, Wauters et al. 1996).

2.4.2 Synthesis of TAFI:

TAFI is encoded by the CPB2 gene that is located on chromosome 13 (13q14.11) (Eaton, Malloy et al. 1991). Nineteen single nucleotide polymorphisms (SNP) have been detected in the CPB2 gene (Boffa, Maret et al. 2008) of which six are located within the coding region. Two of these SNPs produce an amino acid substitution: +505G/A and +1040C/T which give rise to an Ala/Thr substitution at position 147 and Thr/Ile substitution at position 325 respectively (Zhao, Morser et al. 1998; Brouwers, Vols et al. 2001). Only one of these polymorphisms, located at position 325, results in an amino acid substitution which affects TAFI's function by increasing TAFI's half-life two fold (Schneider, Boffa et al. 2002).

TAFI is predominantly synthesised in the liver and is present in plasma as a zymogen (Bouma, Marx et al. 2001). TAFI is also produced in megakaryocytes, but this only accounts for 0.1% of the total circulating TAFI concentration (Declerck 2011). Despite the relatively small amount released by platelets, the local boost of TAFI at the site of thrombus formation has a significant impact on fibrinolysis as only small quantities of TAFI are required to affect clot lysis. The local secretion of TAFI may also account for platelet-mediated resistance to fibrinolysis. Circulating TAFI levels range between 73-275 nm (4-15 $\mu\text{g ml}^{-1}$) (Foley JH 2013). This wide variation has been attributed to: different isoforms of TAFI having differing reactivity to commercially available ELISA assays and altered TAFI gene expression as a result of hormonal control, the action of various disease states and SNPs changing mRNA stability (Gils, Alessi et al. 2003; Boffa MB 2007; Boffa, Maret et al. 2008).

2.4.3 TAFI activation

TAFI is formed of a catalytic domain joined to a N-terminal activation peptide via a linker region (Marx, Brondijk et al. 2008). The zymogen TAFI is converted into its active form (TAFIa) by the proteolytic cleavage of the Arg⁹²-Ala⁹³ bond by either thrombin or plasmin. Thrombin is a weak activator of TAFI. However, when complexed with thrombomodulin (a transmembrane protein expressed on the surface of endothelial cells) its efficacy of TAFI activation is increased 1250 fold (Bajzar, Morser et al. 1996) . Binding to thrombomodulin modulates the substrate specificity of thrombin so that it is no longer recognised by fibrinogen and other substrates. Instead the thrombin-thrombomodulin complex (T/TM) produces activated protein C (APC) an anticoagulant which acts against the 1250 fold increase in TAFI activation to prevent massive coagulation (Declerck 2011). Thus clot formation is localised to the site of

vascular injury. TAFI can also be activated by plasmin, a stronger activator than thrombin (Mao, Cooper et al. 1999). TAFI 's activation by plasmin results in TAFI generation adjacent to fibrin thus preventing premature clot lysis.

2.4.4 Physiological roles of TAFI

2.4.4.1 The attenuation of fibrinolysis:

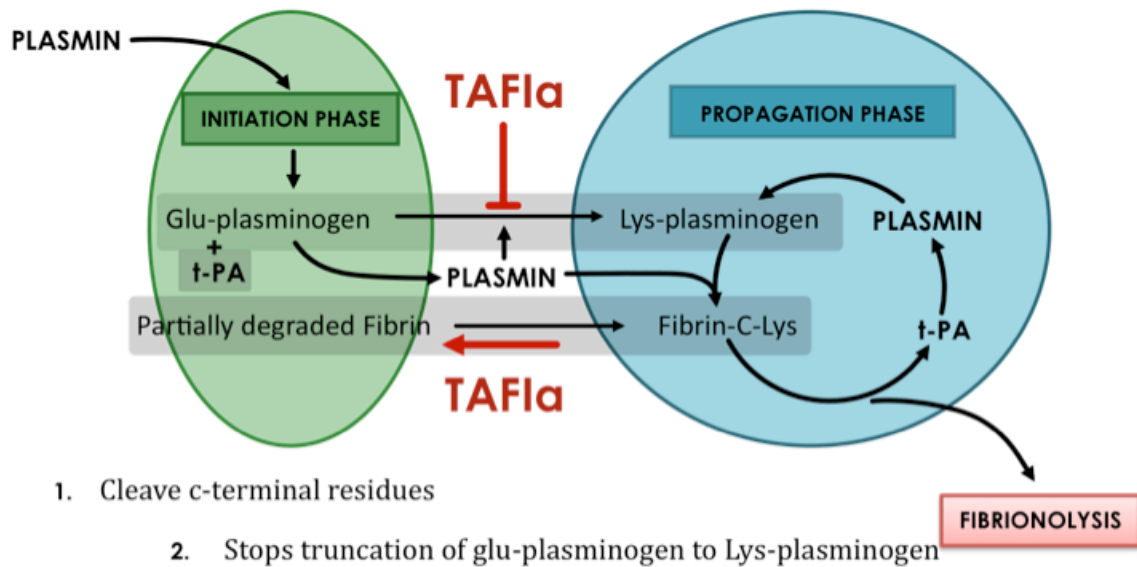
Fibrinolysis is essential for haemostasis and enables the removal of the haemostatic plug upon repair of vascular damage. Furthermore, in conjunction with physiological anticoagulants, it limits pathological propagation of the fibrin clot, thus maintaining vessel patency. Tissue plasminogen activator (t-PA) converts plasminogen to plasmin. This reaction initiates fibrinolysis. The plasmin then acts on the fibrin clot which is partially degraded, leaving exposed C-terminal lysine residues on its surface. These C-terminal lysine residues act to produce a burst of plasmin generation and thus play an important role in the regulation of fibrinolysis. The lysine residues have 4 main actions: i) they bind plasminogen and this accumulation leads to a burst of plasmin generation at the clot surface; ii) the lysine residues induce conformational changes in the structure of plasminogen, making it a better substrate for conversion to plasmin by t-PA (Fleury and Angles-Cano 1991); iii) Plasmin also binds to the lysine residues which protects it from inactivation by α_2 - antiplasmin and iv) partially degraded fibrin acts as a co-factor in plasmin mediated conversion of Glu-plasminogen to Lys-plasminogen, a better substrate for t-PA (Hoylaerts, Rijken et al. 1982). TAFI removes the C-termial lysines from partially degraded fibrin, thus inhibiting fibrin's co-factor role in plasminogen activation. This dramatically decreases the fibrinolytic activity of t-PA.

TAFI function is dependent on its concentration reaching a threshold value determined by plasmin concentration: higher TAFI concentrations are required to inhibit

fibrinolysis with higher plasmin concentrations. Plasmin concentration is determined by local levels of plasminogen activation that are, in turn, regulated by local concentrations of plasminogen activators and inhibitors. Factors that direct TAFIa concentration include: plasma TAFI levels, the extent of their activation and TAFIa's intrinsic stability. The most significant of these factors is the rate of TAFI activation. Large rapid increases in TAFIa levels inhibit fibrinolysis far less efficiently than a small sustained release of TAFIa (Colucci and Semeraro 2012)

TAFIa affects fibrinolysis even when as little as 1% of local TAFI zymogen has been activated. It has been demonstrated that the TAFI zymogen has some enzymatic activity, although with much less catalytic efficiency than TAFIa (Valnikova Z 2007). However, TAFI zymogen has double the substrate affinity than TAFIa, which, in part, may compensate for its reduced catalytic efficiency (Valnikova Z 2007). The zymogen can cleave small substrates but has a limited capability to act on plasmin modified fibrin degradation products (Foley JH 2008).

Figure 3: Role of TAFI in fibrinolysis



2.4.4.2 Role of TAFI in inflammation:

Other substrates for TAFI α have been identified including: bradykinin, thrombin cleaved osteopontin and activated complement factors C3a and C5a (Shinohara, Sakurada et al. 1994; Campbell, Lazoura et al. 2002). This suggests that TAFI's actions are not limited to inhibiting fibrinolysis, but may have a broader role as an anti-inflammatory, affecting cell migration and wound healing. Thrombin can act as either as a pro-coagulant or an anti-inflammatory at sites of inflammation. It achieves the anti-inflammatory actions by binding to thrombomodulin (TM) to activate APC and TAFI α . TAFI α then cleaves kinins, anaphylatoxins and thrombin cleaved osteopontin, rendering them inactive (Myles, Nishimura et al. 2003).

TAFI's role as an anti-inflammatory agent was previously overlooked due to Carboxypeptidase N (CPN) masking TAFI's action on inflammatory mediators

(Skidgel RA 2007). Unlike TAFI, CPN does not require activation (Skidgel RA 2007; Walker JB 2008).

C5a is a potent inflammatory mediator and acts by: recruiting and activating neutrophils (Mollnes TE 2002), increasing vascular permeability (Mulligan MS 1996; Liu ZM 2010) and stimulating smooth muscle contraction (Scheid CR 1983). Additionally, C5a can release tissue necrosis factor (TNF) and histamine from the lysosomal degradation of inflammatory cells (el-Lati SG 1994; Kikuchi Y 2002). A study of C5a activated alveolitis in mice demonstrated significantly decreased inflammation in TAFI deficient mice compared with wild mice (Nishimura T 2007). The same study examined the effects of infusion of mutated thrombin capable of TAFI activation but not clot generation. Wild type mice were further protected against inflammation in direct contrast to TAFI deficient mice (Nishimura T 2007). Through its inactivation of C5a, TAFIa may protect against inflammation.

2.4.5 Instability of TAFIa

TAFIa is rendered inactive by conformational changes that result in the exposure of a cleavage site at Arg³⁰² allowing subsequent proteolytic cleavage by T/TM or plasmin (Marx, Brondijk et al. 2008). TAFIa demonstrates a variable half-life dependent upon temperature. It varies from eight minutes at 37°C to several hours at 22°C (Brouwers, Vols et al. 2001; Schneider, Boffa et al. 2002). TAFIa instability is also affected by the presence of the polymorphism at position 325. When the polymorphism results in an I³²⁵ variant, TAFIa's half-life is doubled (Schneider, Boffa et al. 2002). The I³²⁵ variant has been associated with reduced inflammation in addition to a longer anti-fibrinolytic effect (Schneider, Boffa et al. 2002; Gregersen, Leung et al. 2011). No physiological inactivators for TAFIa have been reported, thus its intrinsic instability contributes to its

regulation, which is supported by the fact that more stable variants have a higher anti-fibrinolytic activity (Ceresa, Peeters et al. 2007).

2.4.6 TAFI inhibitors

TAFIa is inactivated by chelating agents, e.g. EDTA (Willemse, Heylen et al. 2009). Several low molecular weight non specific inhibitors have been identified including reducing agents and small synthetic substrate analogues (Willemse, Heylen et al. 2009). The disadvantage of these inhibitors is that they also inhibit other carboxypeptidases including CPN and pancreatic carboxypeptidase CPB (Willemse, Heylen et al. 2009). More potent selective TAFIa inhibitors are in development (Willemse, Heylen et al. 2009). Potato tuber carboxypeptidase (PTCI) is naturally occurring and competitively inhibits TAFIa (Schneider and Nesheim 2003). PTCI has a biphasic affect: it attenuates clot lysis at low concentrations and enhances lysis at high concentration. The initial attenuation can be explained by the conformational changes in TAFI caused by binding to PTCI. Gradually, large amounts of TAFIa-PTCI complexes accumulate. Thus, when the complexes gradually disassociate rendering TAFI active once more, there is a large local burst of TAFIa. As PCTI does not inhibit CPN it is widely used in clot lysis assays (Schneider and Nesheim 2003).

Attenuating TAFI synthesis and/or activation can also inhibit TAFIa. Several monoclonal antibodies that inhibit TAFI activation have been produced (Gils, Ceresa et al. 2005; Hillmayer K 2008; Buelens K 2010). These monoclonal antibodies either act directly to inhibit TAFIa activity or its activation (Foley JH 2008). Selective TAFIa inhibitors would have several clinical applications. Their use as an adjunct to thrombolytic agents could decrease the dosage needed to be therapeutic. This in turn may decrease bleeding complications associated with thrombolytic therapy. Animal

studies demonstrated significantly lower perfusion times and thrombus weights when PTCI was administered in conjunction with thrombolytic therapy (Klement P 1999) and enhanced thrombolysis when low concentrations of tPA were used with a selective TAFIa inhibitor (Wang YX 2007).

There is conflicting data about the use of TAFI inhibitors as sole agents in the treatment of thrombotic conditions (Foley JH 2008). The administration of PTCI after the formation of arterial or venous thrombosis did not increase fibrinolysis in two animal studies (Klement P 1999; Nagashima M 2000), whereas other studies demonstrated that PTCI and synthetic TAFI inhibitors decreased arterial and venous fibrin deposition and increased lysis of microthrombi (Hashimoto M 2002; Muto Y 2003). In addition, monoclonal antibodies that inhibit TAFIa increased fibrinolysis in e. coli induced sepsis in animal studies (Binette TM 2007). Despite the conflicting data, it is possible to conclude that TAFIa modulates fibrinolysis and the effect produced by TAFI inhibitors is dependant of the timing of administration and the cause of thrombosis.

2.4.7 Pathophysiology of TAFI

Thrombotic disorders may be due to impaired fibrinolysis resulting from increased levels of TAFI, in addition to increased thrombin formation. This is supported by reports of elevated TAFI levels seen in patients with venous thrombosis (Schroeder, Kucher et al. 2003) and men awaiting coronary bypass grafting to treat stable angina pectoris (Silveira, Schatteman et al. 2000). In addition, epidemiological studies have demonstrated elevated TAFI levels in stroke patients (Montaner, Ribo et al. 2003; Santamaria, Oliver et al. 2003). However, there is also data in the literature that do not support the theory that thrombotic events are attributable to high TAFI levels. Several

studies show no difference in TAFI concentration in patients who suffer a myocardial infarction and controls (Juhan-Vague, Morange et al. 2002; Schroeder, Chatterjee et al. 2002; Juhan-Vague and Morange 2003; Salomon, Steinberg et al. 2003). In one large international multicentre study which compared 498 patients who suffered a myocardial infarction to 553 age matched controls, no significant difference in TAFI levels were seen (Juhan-Vague, Morange et al. 2002). Furthermore, fewer patients than controls had a TAFI level greater than the 90th centile (Juhan-Vague, Morange et al. 2002). The different conclusions drawn by these studies may be in part due to the use of different methods to determine TAFI activity/ antigen levels. Isoforms of TAFI demonstrate different reactivity to some of the commercially available ELISAs. Thus, TAFI-I325 (which has greater fibrinolytic effect due to its longer half life) can be underestimated leading to an incorrect overall TAFI activity. Therefore, only well characterised assays which assess TAFI activity rather than antigen levels should be used in further studies.

Table 1: Studies of changes in TAFI Ag and TAFIa in Normal pregnancy and PET

REFERENCE	STUDY DESIGN	NUMBER OF PARTICIPANTS	ASSAYS PERFORMED	RESULTS
(Chetaille, Alessi et al. 2000)	Case control	12 Normal pregnancies 3 rd Trimester Age matched non pregnant controls (n not specified)	TAFI Ag ELISA (Milan Analytica)	Patient group: 95+/- 35 (%) Control Group: 95+/-39 (%)
(Chabloz, Reber et al. 2001)	Cross sectional	144 Normal pregnancies	TAFI Ag ELISA (Milan Analytica)	Data expressed as box plots only, no numerical data. Significant increase in TAFI Ag levels between 10-14 25-40 weeks No significant increase in TAFI Ag after 29 weeks
(Antovic, Rafik Hamad et al. 2002)	Case control	38 PET 16 Normal pregnant controls	TAFI Ag ELISA (Affinity Biologicals)	Patient group: 48.89 mcg/ml (mean) Control Group: 116.3 mcg/ml (mean)
(Alacacioglu, Ozcan et al. 2004)	Case control	30 PET 30 Normal pregnant controls	TAFI Ag ELISA VisuLise™	Patient group: 12.55 +/- 1.88 (mcg/ml) Control group: 12.29+/-3 (mcg/ml)
(Mousa, Downey et al. 2004)	Cross sectional	152 normal pregnancies	TAFI Ag ELISA (Affinity Biologicals)	10-14 weeks: 6.6+/- 1.2 (mcg/ml) 25-29 weeks: 8.2 +/-2.3 (mcg/ml) 35-39 weeks: 9.6 +/- 2 (mcg/ml) within 24 hours of delivery: 7.2 +/- 1.1 (mcg/ml)
(Acosta-Tejeda, Baptista-Gonzalez et al. 2011)	Case control	87 PET 87 Normal pregnant controls	TAFI Ag ELISA (Actichrome)	Patient group: Median (range) 20.4 mcg/ml (17.3-23.5 Control group: Median (range) 13.3 mcg/ml(12.06-14.
(Watanabe, Wada et al. 2001)	Cross sectional	176 Normal pregnancies	TAFIa TAFIa Acivity Kit (American diagnostica)	6-11 weeks: 2.57 +/- 1.05mcg/ml 18-22 weeks: 3 +/- 0.99 mcg/ml 28-32 weeks: 3.07 +/- 0.99mcg/ml 37-39 weeks: 3.21 +/- 0.92mcg/ml 12-36 hours post delivery: 2.69 +/- 1.17mcg/ml
(Zhang, Hu et al. 2008)	Case control	10 PET 30 Normal pregnant controls	TAFI Ag ELISA (American Diagnostica)	Patient group: 1 st trimester 106.64+/- 19 (%) 2 nd trimester 129.23 +/-25.05 (%) 3 rd trimester 139.14 +/- 30.12 (%) Control group: 1 st trimester 85.35+/- 24.69 (%) 2 nd trimester 99.65 +/- 18.27 (%) 3 rd trimester 110.12+/- 23.36 (%)

2.4.8 TAFI in pregnancy

2.4.8.1 TAFI in normal pregnancy:

There are contrasting reports of TAFI levels during pregnancy (Chetaille, Alessi et al. 2000; Chabloz, Reber et al. 2001; Watanabe, Wada et al. 2001; Mousa, Downey et al. 2004). Three out of the four published studies considering TAFI Ag and TAFIa in normal pregnancy, demonstrate a significant progressive increase from early pregnancy (Chabloz, Reber et al. 2001; Watanabe, Wada et al. 2001; Mousa, Downey et al. 2004). Chabloz et al performed a cross sectional cohort study assessing TAFI Ag and D-dimer levels in 144 women with uncomplicated singleton pregnancies (Chabloz, Reber et al. 2001). They reported a steady significant increase in TAFI Ag levels between 10 and 25 weeks gestation, after which, TAFI Ag levels plateaued until they started to fall after delivery (Chabloz, Reber et al. 2001). As Chabloz et al did not include any non-pregnant controls, the study could not confirm if TAFI Ag levels were increased when compared to outside of pregnancy.

A more recent cross sectional case control study by Watanabe et al focused on changes in TAFIa during pregnancy (Watanabe, Wada et al. 2001). This prospective study demonstrated significantly higher TAFIa in 176 women with normal pregnancies compared to 15 non-pregnant women and confirmed a progressive increase in TAFIa starting in the first trimester, continuing up to 20 weeks after which levels remained steady until they started to decrease postnatally (Watanabe, Wada et al. 2001). This is one of the few studies which examines TAFI activity in normal pregnancy (Watanabe, Wada et al. 2001).

Another cross sectional study of 183 normal pregnancies measured TAFI Ag levels at four weekly intervals and reported a progressive increase in TAFI Ag throughout pregnancy, maximal at 35-39 weeks gestation, and a gradual decline in the levels post delivery (Mousa, Downey et al. 2004). Although it did not report changes in TAFIa, this study did examine TAFI's functional effect by measuring CLT, both with and without the addition of a TAFI inhibitor. The changes in CLT mirrored those of TAFI Ag, increasing with gestation. Thus, there is a progressive impairment of fibrinolysis as pregnancy progresses. Addition of the TAFI inhibitor during the CLT assay abrogated the increase in CLT, suggesting that the decreased rate of fibrinolysis is attributable to TAFI (Mousa, Downey et al. 2004).

In contrast to the three studies previously described, Chetaille et al reported that pregnancy had no influence on TAFI Ag levels (Chetaille, Alessi et al. 2000). This large cross sectional study examined TAFI Ag levels in 249 healthy subjects (139 males, 110 non pregnant females and 12 pregnant women in their third trimester) (Chetaille, Alessi et al. 2000). Neither gender, nor pregnancy had any influence on mean TAFI Ag levels (Chetaille, Alessi et al. 2000). The discrepancy between this and other studies is most likely attributable to the small sample size and the large inter-individual variation in TAFI Ag levels (Chetaille, Alessi et al. 2000).

2.4.8.2 TAFI in Pre-eclampsia:

A handful of studies have investigated TAFI levels in PET and have produced conflicting reports (Antovic, Rafik Hamad et al. 2002; Alacacioglu, Ozcan et al. 2004; Dusse, Cooper et al. 2007; Zhang, Hu et al. 2008; Martinez-Zamora, Tassies et al. 2010; Acosta-Tejeda, Baptista-Gonzalez et al. 2011). Antovic et al performed a case control

study to evaluate the role of impaired fibrinolysis in PET and IUGR (Antovic, Rafik Hamad et al. 2002). The study measured third trimester levels of TAFI Ag, in 46 women with PET (eight of whom also had IUGR) and 16 normal pregnant controls. The study reported significantly lower mean TAFI Ag levels in the patient group (49 compared to 116 in the control group) (Antovic, Rafik Hamad et al. 2002). The study also measured CLT and overall fibrinolytic potential in 15 women in the patient group and 10 controls. In 10 (67%) women from the patient group, clot lysis was not achieved after two hours, compared to no women in the control group. Furthermore, in the remaining five (33%) women with PET, CLT was significantly longer than in the control group. However, the difference in mean CLT was not statistically different between the two groups. Overall fibrinolytic potential was significantly lower in the patient group. However, addition of PTCI (a TAFI inhibitor) did not increase the overall fibrinolytic potential in the patient group. Thus, it can be concluded that although fibrinolysis was impaired in women with PET and/ or IUGR, TAFI Ag levels did not contribute to the situation (Antovic, Rafik Hamad et al. 2002). The low TAFI levels in PET were attributed to impaired liver function and increased urinary excretion (Antovic, Rafik Hamad et al. 2002). However, to confirm the role of renal and hepatic impairment, urinary TAFI Ag levels would have to be measured. This study is also limited by its small sample size and by the fact that only TAFI Ag levels and not activity were measured. Furthermore, the study only included women in their third trimester. Alacacioglu et al performed a similar case control study involving 30 women with PET and 30 pregnant controls in their third trimester, and found that despite a trend for marginally higher TAFI Ag levels in pre-eclamptic women, there was no significant difference in TAFI Ag levels between the two groups (Alacacioglu, Ozcan et al. 2004). The authors speculated that the underlying reason for the difference in their findings

from Antovic et al was the severity of PET in their patient group. Only 7% of their patient group suffered from severe PET, compared to 63% in Antovic et al (Antovic, Rafik Hamad et al. 2002). Thus, their degree of hepatic and renal impairment was lower than in the previous study. Again, the findings of this study may not have achieved statistical significance due to its small sample size. Moreover, only TAFI Ag was reported, again, solely in the third trimester (Alacacioglu, Ozcan et al. 2004).

More recent studies have demonstrated higher TAFI Ag levels in pre-eclamptic women than in normal controls (Zhang, Hu et al. 2008; Martinez-Zamora, Tassies et al. 2010; Acosta-Tejeda, Baptista-Gonzalez et al. 2011). Zhang et al reported results from 10 women with PET, 12 with GHT and 30 normal controls. Samples were collected in each trimester. The study demonstrated significantly higher TAFI Ag levels in women with PET when compared to women with gestational hypertension (GHT) and normal pregnant controls at all gestations (Zhang, Hu et al. 2008). However, there was no significant difference in TAFI Ag levels between women with GHT and the control group. Importantly, this study demonstrated that significantly higher mean TAFI Ag levels were evident in the PET group from the first trimester, prior to the manifestation of clinical symptoms (Zhang, Hu et al. 2008). Thus TAFI could be used as a marker to identify women at risk of PET enabling improved antenatal surveillance and prompt treatment.

Martinez-Zamora et al investigated the changes in CLT and TAFI Ag levels in 76 normal pregnant controls and 82 women with PET (Martinez-Zamora, Tassies et al. 2010). The study also examined TAFI Ag levels and CLT at six weeks postpartum. Amongst the control group there was a progressive increase in TAFI Ag levels from the first to the third trimester, which then dropped after delivery. There was no statistically significant difference in TAFI Ag levels or CLT between the patient and

control groups during pregnancy. However, women with PET had significantly higher postpartum TAFI Ag levels and longer CLT than the controls, thus demonstrating impaired fibrinolysis after delivery (Martinez-Zamora, Tassies et al. 2010).

The most recent study to date evaluated the role of TAFIa in PET (Acosta-Tejeda, Baptista-Gonzalez et al. 2011). In this case control study, TAFIa levels amongst 174 women (87 women with PET and 87 normal controls) were measured after 20 weeks gestation. Mean TAFIa levels were significantly higher in the PET group compared to the controls (20.4 µg/ml vs 13.3 µg/ml p=0.003). In addition, the study also examined if TAFI SNP (G505A, C1040T and G-438A) were associated with PET. The allele frequency and genotype of the polymorphisms were not associated with an increased risk of PET (Acosta-Tejeda, Baptista-Gonzalez et al. 2011). This suggests that the increased levels of TAFIa described were attributable to PET rather than the presence of different polymorphisms.

Increasingly, data available in the literature supports the view that TAFI is further increased from normal pregnancy levels in conditions including PET and IUGR (Antovic, Rafik Hamad et al. 2002; Acosta-Tejeda, Baptista-Gonzalez et al. 2011). If this is confirmed, it may be the case that anti-TAFI agents may have a role in the treatment of these conditions.

2.4.8.3 TAFI in Recurrent miscarriage and implantation failure

Miscarriage is the most common complication of pregnancy and affects 15% of women, most frequently during the first trimester (Clark, Coulam et al. 2001). The majority of

these losses are sporadic and do not recur. However, 2-5% of women experience recurrent miscarriages (RM)(Clark, Coulam et al. 2001). Repetitive pregnancy loss suggests an underlying cause and there has been a great deal of interest in defining a mechanism of RM. Unfortunately, a cause is only isolated in 50% of RM patients (430 2009)Li TC et al Hum Reprod Update 2002;8:463-81).

It has been proposed that recurrent implantation failure (RIF) following embryo transfer (ET) in IVF is part of the spectrum of RM and that the two represent different manifestations of the same disease process (Martinez-Zamora, Creus et al. 2011). Indeed, both RIF and RM can be a result of embryonic chromosomal abnormalities or the same abnormalities in maternal endocrine, immunological or haemostasis (Christiansen, Nielsen et al. 2006; Stern and Chamley 2006). Five percent of women in their first miscarriage and 30-40% of women undergoing IVF who subsequently miscarry will have a karyotypically normal pregnancy (Clark, Coulam et al. 2001). This increases the chance of RM or recurrent implantation failure (RIF) (Boue et al Teratology 1973;12:11-26) and suggests an underlying cause other than embryonic chromosomal abnormality. It has been proposed that alterations in maternal haemostasis result in thrombosis of the uteroplacental vessels causing recurrent reproductive failure (Rai 2003).

Several studies have investigated the role of TAFI in RM and RIF (Folkeringa, Korteweg et al. 2009; Knol, Veeger et al. 2009; Masini, Ticconi et al. 2009; Martinez-Zamora, Creus et al. 2010; Martinez-Zamora, Tassies et al. 2010; Pruner, Djordjevic et al. 2010; Martinez-Zamora, Creus et al. 2011; Colucci and Semeraro 2012; Legnani,

Bovara et al. 2012) producing conflicting reports. Several studies report lower levels of TAFI in women with RM when compared to controls (Folkeringa, Korteweg et al. 2009; Masini, Ticconi et al. 2009; Legnani, Bovara et al. 2012) and go on to suggest that higher TAFI levels confer a protective effect against RM (Knol, Veeger et al. 2009).

Legnani et al performed a case control study including 140 women with a history of RM and 140 controls (Legnani, Bovara et al. 2012). They reported significantly lower TAFIa levels in women with either three or more consecutive losses or two miscarriages with normal karyotyping in at least one pregnancy. Women in the control group had no history of miscarriage. The authors proposed that enhanced fibrinolysis was detrimental to implantation. This theory was supported by evidence of higher rates of RM in women with Factor XIII deficiency, afibrinogenaemia and dysfibrinogenaemia, all conditions which demonstrate inadequate or defective fibrin (Inbal and Muszbek 2003) . In addition, hypofibrinolysis is associated with lower levels of fibrin degradation products which induce apoptosis in placental trophoblast cells (Guo, Hernandez et al. 2009). TAFIa has been shown to inactivate bradykinin, C3a and C5a in vivo (Myles, Nishimura et al. 2003). The authors also suggested that TAFIa's role in reducing inflammation may play a role in the pathophysiology of RM (Legnani, Bovara et al. 2012).

There are several limitations to this study. In addition to the limited sample size and retrospective design, the control group included women who had never been pregnant. Thus, it is unknown if they had they would be able to become pregnant, or go on to have a live birth.

Folkeringa et al performed a retrospective study examining TAFIa levels in 843 women (Folkeringa, Korteweg et al. 2009). They divided the cohort into two groups: those with elevated TAFIa and those with normal levels and found no significant difference in rates of miscarriage between the two groups, (22.5% vs 27.9% respectively $p=0.128$) (Folkeringa, Korteweg et al. 2009). Further sub-analysis revealed significantly reduced rates of recurrent miscarriage in women with high TAFIa (3.8% vs 7.9% $p=0.041$) (Folkeringa, Korteweg et al. 2009). This study is limited in several ways. Firstly, its retrospective design introduces the possibility of recall bias regarding fetal loss. Furthermore, the study group contains women who have experienced only one miscarriage making it more likely that aneuploidy was the cause of pregnancy loss; women with known thrombotic disorders were not excluded and none of the participants were investigated for other causes of miscarriage. The authors do not clarify their definition of RM (two or more consecutive losses or three or more).

A case control study by Masini et al reviewed 86 women who suffered two or more miscarriages and 72 women who had at least two children and no personal experience of miscarriage (Masini, Ticconi et al. 2009). They reported a significantly higher frequency of the genotypes +505A/A and +1583A/A in the control group (Masini, Ticconi et al. 2009). These genotypes have been seen to result in higher TAFI Ag levels (Frere, Morange et al. 2005). The +505G/G genotype which correlates with lower TAFI Ag levels (Frere, Morange et al. 2005) and was more commonly seen in women with RM (Masini, Ticconi et al. 2009). Although the authors defined RM as two or more losses, a sub analysis revealed the same findings when the RM group were

stratified according to the number of miscarriages that they had experienced (two or three or more) (Masini, Ticconi et al. 2009).

Masini et al's findings were supported by a further genetic case-control study which revealed significantly higher frequency of the +1040T/T genotype in women with RM (Pruner, Djordjevic et al. 2010). However, neither of these genetic studies were accompanied by the parallel quantification of TAFIa or TAGFI Ag levels (Masini, Ticconi et al. 2009; Pruner, Djordjevic et al. 2010).

In contrast to the aforementioned genetic studies (Masini, Ticconi et al. 2009; Pruner, Djordjevic et al. 2010), Martinez –Zamora et al found no significant difference in the distribution of TAFI polymorphisms case control studies of women with unexplained RM (Martinez-Zamora, Creus et al. 2010) or women with recurrent implantation failure (RIF) after IVF (Martinez-Zamora, Creus et al. 2011).

The variation in TAFI Ag levels in the normal population can in part be attributed to genetic factors, but the relationship between TAFI SNPs, antigen levels and the risk of thrombotic complications remains poorly understood (Gris, Schved et al. 1990; Rai, Tuddenham et al. 2003).

Two studies revealed significantly higher levels of TAFI Ag and longer CLT in women with reproductive failure when compared to controls (Martinez-Zamora, Creus et al. 2010; Martinez-Zamora, Creus et al. 2011). The first investigated 119 women with RM and 64 controls (Martinez-Zamora, Creus et al. 2010) and the second 30 women with RIF after IVF and 60 controls (Martinez-Zamora, Creus et al. 2011). The women in

the control groups of both studies had had a live birth after an uncomplicated pregnancy and no history of miscarriage. All the RM patients had suffered three or more losses and all other causes of RM (including thrombotic disorders) were excluded (Martinez-Zamora, Creus et al. 2010).

Women with RM are in a prothrombotic state even outside of pregnancy (Regan and Rai 2002; Rai 2003; Sebire, Backos et al. 2003). As yet, it is not understood how thrombophilic factors impact on RM and RIF.

Ovulation, implantation and placentation are all reliant on haemostatic mechanisms, with the fibrinolytic system regulating early trophoblastic invasion. (Briefly), plasmin both activates matrix metalloproteinases and degrades certain parts of the extracellular matrix of the decidua to allow trophoblastic invasion (Lala and Chakraborty 2003). It follows that altered fibrinolysis results in decreased trophoblastic invasion.

This provides a biological basis for disordered fibrinolysis' role in reproductive failure. Previous studies suggest that hypofibrinolysis may be involved in early pregnancy loss (Gris, Schved et al. 1990; Coulam, Jeyendran et al. 2006; Sotiriadis, Makrigiannakis et al. 2007). The two studies by Martinez Zamora et al (Martinez-Zamora, Creus et al. 2010; Martinez-Zamora, Creus et al. 2011) demonstrated a significant direct relationship between CLT and TAFIag levels across all their study populations which lead to the conclusion that the impaired fibrinolysis seen in women with reproductive failure can be attributed to increased TAFIag levels.

Both studies demonstrating increased levels of TAFIag in women with reproductive failure had stringent inclusion criteria (Martinez-Zamora, Creus et al. 2010; Martinez-Zamora, Creus et al. 2011). All women in the affected groups had suffered at least three miscarriages/ implantation failures who had had all other causes of RM/ RIF excluded. In addition, these studies included CLT to reflect global haemostasis (Martinez-Zamora, Creus et al. 2010; Martinez-Zamora, Creus et al. 2011). This is in contrast to studies that found lower levels of TAFIa or a higher frequency of SNPs associated with lower TAFIag levels, in women with miscarriage (Folkeringa, Korteweg et al. 2009; Masini, Ticconi et al. 2009; Pruner, Djordjevic et al. 2010; Legnani, Bovara et al. 2012).

Recent RCTs have not been able to demonstrate a clear benefit in the use of low molecular weight heparin or low dose aspirin to prevent unexplained RM (Laskin, Spitzer et al. 2009; Clark, Walker et al. 2010; Kaandorp, Goddijn et al. 2010). This would imply that fibrinolysis is not critical to implantation. However, heparin can not stimulate fibrinolysis through TAFI dependant mechanisms (Colucci, Pentimone et al. 2002) and chronic high doses of 650mg 12 hourly are required to clot lysis susceptibility (bjornsson, Schneider et al. 1989) . Thus it is reasonable to conclude that TAFIs contribution to enhanced fibrinolysis has a role in the pathophysiology of early pregnancy loss.

In conclusion, there are contradictory reports of TAFI levels in women with early reproductive failure. This is compounded by the fact that different definition of recurrent miscarriage are used in addition to different methods of quantifying TAFI. Further large multicentre studies are warranted to clarify TAFI's role in RM.

2.5 Pre eclampsia

Pre eclampsia (PET) is a multisystem disorder of pregnancy which affects 2-8% of all pregnancies (Ismail and Higgins 2011). It is a major cause of maternal and fetal morbidity and mortality. In the UK, PET and eclampsia continue to be one of the leading direct causes of maternal death with a rate of 0.85/100,000 maternities between 2003 and 2005 (Cantwell, Clutton-Brock et al. 2011). Worldwide, 10-15% of all maternal deaths are a direct result of PET or eclampsia (Duley 2009). Infants born of pre-eclamptic mothers are five times more likely to die (Lain and Roberts 2002).

It is a systemic syndrome resulting from inadequate trophoblastic invasion of the spiral arteries during placentation. This leads to widespread maternal endothelial dysfunction resulting in subsequent hypertension, proteinuria and systemic manifestations of PET. When left untreated, PET can result in severe maternal complications including eclampsia, stroke, renal failure, pulmonary oedema and liver capsule rupture all of which can be fatal (Mol 2016). PET can also affect the fetus as a result of intrauterine growth restriction (IUGR) and premature delivery. Offspring of mothers with PET are at increased risk of cerebral palsy and respiratory distress as a consequence of being small for gestational age and being delivered prematurely, either iatrogenically or spontaneously (Hansen AR 2010; Strand KM 2013). In addition to its immediate effects, PET can also result in long term consequences including: decreased health related quality of life; increased post partum depression and increased maternal cardiovascular health risks (Blom EA 2010; Cusimano MC 2014; Prick BW 2015)

2.5.1 Definition of PET:

The International Society for the Study of Hypertension in Pregnancy (ISSHP) changed the diagnostic criteria for PET in 2014 to de-novo hypertension occurring after 20 weeks gestation associated with either proteinuria of greater than 300mg/ day; any other maternal organ dysfunction (including renal or liver compromise, and haematological or neurological complications) or placental insufficiency (Tranquilli AL 2014).

As proteinuria is no longer essential to diagnose PET, proteinuric and non-proteinuric PET are categorised separately.

Clinically, hypertension is defined as greater than or equal to 140 mmHg systolic and/or 90 mmHg diastolic measured on two occasions at least four hours apart after 20 weeks gestation (Roberts and Gammill 2005; (NICE) 2011). PET superimposed on pre-existing essential hypertension can be diagnosed if a woman develops significant proteinuria, organ dysfunction or uteroplacental insufficiency after 20 weeks (Mol 2016). Significant proteinuria is defined as a protein concentration of ≥ 30 mg ($\geq 1+$ on urine dipstick) present in a minimum of two urine samples taken at least 4-6 hours but no more than seven days apart.

2.5.1.2 Definition of Severe PET

The National Institute for Health and Care Excellence (NICE) define severe PET as severe hypertension (a systolic blood pressure of greater or equal to 160 mmHg and/ or a diastolic blood pressure of greater than or equal to 110mmHg) with or without symptoms and/ or deranged biochemical or haematological indices ((NICE) 2011).

The American College of Obstetrics and Gynaecology (ACOG) consider PET to be severe in women with any of the features listed in Table 1. Although the ACOG's

definition of severe PET is useful to stratify patients, it does not address superimposed PET. This can develop in the context of pre-existing maternal disease and is a situation frequently encountered in clinical practice. The ISSHP have overcome this issue by further categorising the hypertensive disorders of pregnancy into chronic hypertension (noted prior to 20 weeks gestation), Gestational hypertension (hypertension after 20 weeks with no associated proteinuria) and PET (hypertension after 20 weeks associated with proteinuria (Tranquilli AL 2014).

2.5.1.3 Early and late onset PET

The outcome of PET has been shown to vary with the timing of onset of the disease (Vatten LJ 2004). It has been proposed that there are different pathophysiological processes underlying early onset PET (prior to 34 weeks) and late onset PET (after 34 weeks gestation) (Raymond D 2011). Early onset disease tends to be more severe and causes higher levels of maternal morbidity and offspring are more likely to have lower birth weights (Vatten LJ 2004). Adverse maternal outcomes are recorded in 10% of women with PET, with the risk increasing to 15% with early onset disease (von Dadelszen P 2011).

Table 2: ACOG Criteria for Severe PET

New onset hypertension (blood pressure \geq 140/90) in a previously normotensive woman after 20 weeks gestation with a urinary protein of 0.3g or more in 24 hours with one or more of the following features
<ul style="list-style-type: none">• Blood Pressure: Systolic BP \geq 160 mmHg and / or Diastolic BP \geq 110 mmHg measured 6 hours apart with the patient on bed rest• \geq 5g proteinuria in 24 hours or 3+ or greater on two random urine dips performed at least 4 hours apart• Urine output of less than 500ml in 24 hours• Headaches or visual disturbances• Right upper quadrant or epigastric pain• Deranged liver function tests• Thrombocytopenia• Evidence of fetal growth restriction

2.5.2 Maternal morbidity and mortality

PET represents one of the leading causes of maternal mortality both in the UK and worldwide. PET is currently the fifth most common cause of direct maternal death (a death directly due to a complication of pregnancy) and account for 8.7% of direct deaths between 2011-2013 (Knight M 2015). In the developing world, the incidence of PET is seven fold higher than in developed countries and accounts for 12% of direct maternal deaths: 63 000 women died from the disease in 2000 (Centre for Maternal and Child

Enquiries) ((CMACE) 2011). PET also contributes to maternal morbidity, accounting for one third of significant morbidity (Waterstone M 2001) with 5% of sufferers requiring admission to intensive care (Tuffnell DJ 2005).

2.5.3 Risk Factors

Risk factors for PET include a prior history of the disease (particularly those women who had early onset PET) (Barton and Sibai 2008) and a family history of PET in a first degree relative which increases the risk of severe PET by up to fourfold (Carr, Epplein et al. 2005). A history of PET in the paternal grandmother also confers an increased risk (Esplin, Fausett et al. 2001).

Conversely, smoking decreases the risk of PET (England, Levine et al. 2002) as does a previous miscarriage with the same partner and a high fruit intake (North RA 2011). Moderate risk factors include maternal age over 40; body mass index greater than 35; polycystic ovarian syndrome and a pregnancy interval of longer than 10 years ((NICE) 2011; Roos N 2011). Unfortunately, these risk factors only predict 30% of women who become pre-eclamptic (Leslie K 2011).

2.5.3.1 Parity and Paternity

The disease most commonly occurs in healthy nulliparous women in whom the incidence has been reported to be 7.5% (Young, Levine et al. 2010), making nulliparous women three times more likely to develop the disease than parous women (Duckitt and Harrington 2005). However, a change in paternity or a prolonged interval between pregnancies puts multiparous women at similar risk of PET to primiparous women (Tubbergen, Lachmeijer et al. 1999).

There is a higher incidence of PET in women with limited exposure to paternal sperm prior to the index pregnancy. A previous child or a pregnancy loss with the same partner decreases the risk of PET. However, the protective effect of a previous pregnancy is lost with a new partner (Broughton-Pipkin F 1994). Men who have fathered previous pregnancies complicated by PET are twice as likely to father a pre-eclamptic pregnancy in a different woman, regardless of their parity (Lie RT 1998).

2.5.3.2 Maternal Age

Extremes of maternal age are frequently attributed as a risk factor for the development of PET. It has been suggested that younger women are at greater risk due to their limited exposure to sperm prior to their pregnancy. However, Duckitt et al reported that women under the age of 17 were not at a significantly increased risk of PET (Duckitt and Harrington 2005). In contrast, maternal age over 40 years is a widely accepted risk factor for PET, irrespective of parity (Broughton-Pipkin F 1994; (NICE) 2011).

2.5.3.3 Ethnicity

There are established links between ethnicity and PET, with black women at increased risk and Asian women at lower risk compared to the Caucasian population (Caughey AB 2005). Black race also increases the likelihood of recurrence of PET in future pregnancies (Wright D 2012).

2.5.3.4 Medical Co-morbidities

Pre-existent maternal co-morbidities can also act as risk factors for PET. These include: hypertension; diabetes mellitus; autoimmune disorders including systemic lupus

erythematosus and antiphospholipid syndrome; chronic renal disease and hypercoaguable states (Duckitt and Harrington 2005; Barton and Sibai 2008). The common theme connecting these conditions is their pathological effect on endothelium and increased systemic inflammation.

Table 3: Medical Conditions associated with PET

Condition	Increase in PET risk	Reference
Diabetes Mellitus	15-18%	((NICE) 2011)
Thrombophilia	Five fold increase	(Kingdom J 2011)
Chronic Renal disease	Three fold increase	(Vellanki 2013)
Systemic Lupus Erythematosus	Two fold increase	(Paez MC 2013)

2.5.3.5 Increased placental mass

Conditions which increase placental mass including: multiple gestation (Duckitt and Harrington 2005; Bdolah, Lam et al. 2008) and hydatiform mole (Koga, Osuga et al. 2010) have been reported as risk factors for PET. It has been suggested that increased placental mass induces a greater degree of inflammation during the first trimester (Redman, Sacks et al. 1999). A primigravidae with a twin pregnancy has a five fold

increased risk of PET and a multiparous woman has a 10 fold increased risk regardless of her parity, compared to a primigravidae with a singleton pregnancy (Macgillivray I 1988).

2.5.3.6 Obesity

There is a clear link between obesity and PET which has been confirmed in studies controlled for other risk factors (O'Brien TE 2003). For every 5-7 kg/m² increase in body mass index (BMI) the incidence of PET doubles (O'Brien TE 2003). Dekker et al postulated several possible explanations for the link between obesity and PET including the hyperdynamic circulation and dyslipidaemia seen in obesity, cytokine-mediated oxidative stress and hyperinsulinaemia leading to increased sympathetic activation and increased tubular sodium desorption (Dekker G 2001).

2.5.3.7 Assisted Conception

Generally, women seeking fertility treatment are older, have a higher BMI and more likely to have significant co-morbidities or underlying conditions such as polycystic ovarian syndrome. Thus, the cohort is at increased risk of developing PET. Assisted reproductive techniques are also an independent risk factor for PET. Egg, semen and embryo donation can affect the fetal-maternal immune interaction. Additionally, assisted reproductive techniques increase the chance of a multiple conception, an independent risk factor for PET (Sibai, Dekker et al. 2005)

2.5.4 Cardiovascular Risk after PET

There is overwhelming evidence in the literature that demonstrates that women with PET are at increased risk of developing cardiovascular disease in later life (Epstein

1964; Sattar N 1997; McDonald SD 2008; Doyle LW 2009; Kvehaugen AS 2011; Cusimano MC 2014) and until very recently this patient group had not been offered preventative measures. Women who have hypertensive disorders during pregnancy demonstrate increased cardiovascular risk as early as two years after delivery: 30% of women who have PET at term have chronic hypertension and 25% will have metabolic syndrome (Hermes W 2013; Veerbeek JH 2015). This then predisposes to vascular endothelial dysfunction and may be the underlying mechanism of increased cardiovascular risk. Alternatively, it may be that pregnancy reveals a subclinical condition that re-emerges at a later time. Patient education provides (them with the) motivation to modify their lifestyle through maintaining a healthy BMI and smoking cessation which can reduce their cardiovascular risk by 4-13% (Berks D 2013). It also enables clinicians to monitor at risk women.

2.5.5 Clinical features:

The spectrum of presentation and lack of a reliable diagnostic test can make diagnosing PET challenging. The majority of women are asymptomatic and are diagnosed due to routine antenatal screening. The cardinal features of PET are a systolic blood pressure (SBP) ≥ 140 mmHg or a diastolic blood pressure (DBP) ≥ 90 mmHg associated with significant proteinuria (>0.3 g in 24 hours). However, these criteria are unhelpful in atypical presentations or PET superimposed on chronic hypertension or renal disease. Furthermore, up to 20% of women present atypically with little or no proteinuria (Sibai, Ramadan et al. 1993). Typically, PET is classified into mild or severe presentations as they require different management. Eclampsia, is defined as a convulsive episode or other signs of altered consciousness and complicates up to 2% of cases (Altman, Carroli et al. 2002). Typically, eclampsia is superimposed on PET. However, 20% of women

with eclampsia do not have associated proteinuria (Noraihan, Sharda et al. 2005) and 33% present postpartum with no prior signs or symptoms of PET (Sibai 2005). Another manifestation of severe PET is Haemolysis, elevated liver enzymes and low platelet (HELLP) syndrome. The onset of HELLP is frequently acute and one in three cases present prior to 28 weeks gestation (Habli M 2009).

PET can also affect the fetus with severe PET resulting in: IUGR, oligo hydramnios, iatrogenic premature delivery and an increased risk of perinatal death (Sibai, Dekker et al. 2005). The pathogenesis of these complications remains unconfirmed, but are thought to be a result of vascular abnormalities in the utero-placental unit which cause placental abruption and infarction (Young, Levine et al. 2010).

In addition to its acute complications, PET is associated with a twofold increased risk of premature maternal cardiovascular and cerebrovascular disease (Young, Levine et al. 2010). Women with PET are ten times more likely to develop chronic hypertension within seven years of their pregnancy compared women who have had uncomplicated pregnancies (Young, Levine et al. 2010). The offspring of pre-eclamptic pregnancies also have an increased risk of cardiovascular disease and metabolic syndrome in later life (Barker, Martyn et al. 1993; Eriksson, Forsen et al. 2001; Osmond, Kajantie et al. 2007).

2.5.5.1 Signs and symptoms:

Clinicians routinely enquire about symptoms of PET including headache, visual disturbances and right upper quadrant or epigastric pain. Unfortunately, no symptoms adequately predict adverse maternal outcomes (Thangaratinam S 2011). Similarly a mean arterial pressure of greater than 140 mmHg or a blood pressure of greater than

170/110 mmHg have limited value in the prediction of eclampsia, placental abruption, or renal, neurological or hepatic impairment (Thangaratnam S 2011).

2.5.6 Pathogenesis of PET:

In normal pregnancy, extravillous cytotrophoblasts originating from the fetus invade the uterine spiral arteries. The fetal cytotrophoblast replaces the endothelium of the maternal spiral arteries. This converts the spiral arteries from small calibre high resistance vessels into low resistance, high capacity vessels to facilitate the increased blood flow and oxygenation required by the fetus. In PET, there is inadequate invasion of the myometrium by the cytotrophoblast, which only penetrates as far as the decidua so the myometrial segments of the spiral arteries remain high resistance vessels which results in fetal hypoperfusion. This is followed by widespread maternal endothelial dysfunction which produces the clinical symptoms of the disease. However, the cause of placental dysregulation remains unknown. Previous research has postulated that hypoxia, inflammation, antiangiogenic proteins, alterations of the renin- aldosterone-angiotensin II pathway, oxidative stress, and genetic factors may all contribute to the pathogenesis of PET (Young, Levine et al. 2010).

2.5.6.1 Role of the placenta:

PET cannot occur without the placenta, whereas the presence of the fetus is irrelevant. Cases of PET have been reported in women with hydatiform mole (where there is no fetus present). Furthermore, the condition was seen to remit after curettage to remove the abnormal placental tissue (Koga, Osuga et al. 2010). Postpartum eclampsia can occur as a result of retained placental fragments and again, symptoms resolve after uterine curettage (Matsuo, Kooshesh et al. 2007). Finally, case reports of PET

complicating abdominal pregnancy report that delivery of the fetus alone did not lead to resolution of PET. This does not occur until the placenta was delivered (Shembrey and Noble 1995).

Severe PET is associated with placental hypoperfusion and ischaemia. Acute atherosclerosis, and diffuse vascular obstruction resulting from fibrin deposition, intimal thickening and necrosis and atherosclerosis have all been found in placentas of women who suffered from severe PET (Young, Levine et al. 2010). Placental infarcts due to occlusion of the spiral arteries are another common pathological feature of severe PET (Salafia, Pezzullo et al. 1998). The severity of placental pathology has been shown to correlate with the severity of the disease (Salafia, Pezzullo et al. 1998). A decrease in uteroplacental perfusion can be detected by uterine artery Doppler ultrasound and frequently precedes the manifestation of clinical symptoms (North, Ferrier et al. 1994).

2.5.6.2 Vascular development of the placenta:

During normal placentation, fetal cytotrophoblasts undergo a process called pseudovasculogenesis in order to function as endothelial cells. This involves the down regulation of adhesion molecules that are typically expressed by the cytotrophoblast as they adopt a vascular phenotype (Zhou, Fisher et al. 1997). In pre-eclamptic patients, the cytotrophoblast adhesion molecules fail to convert to endothelial cell surface adhesion molecules, which inhibits the invasion of the myometrial spiral arterioles (Zhou, Damsky et al. 1997).

2.5.6.3 Altered angiogenic balance:

Angiogenic factors including vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) and their receptors (VEGFR 1, VEGFR 2, Tie 1 and Tie 2) are essential for normal placental vascular development and are expressed by the placenta in the first trimester. Alterations in the regulation or signalling of angiogenic factors or their receptors during early placentation may result in inadequate endovascular invasion. Immunohistochemistry has shown that the expression of VEGF and its receptors is altered in PET (Zhou, McMaster et al. 2002).

VEGF acts to stabilise endothelial cells in mature blood vessels and its actions are particularly important in the liver, kidneys and brain (Maynard, Min et al. 2003; Maharaj, Walshe et al. 2008). FMS-like tyrosine kinase (Flt-1) is a major VEGF receptor. sFlt-1 is a truncated variant of Flt-1 which lacks its transmembrane and intracellular signalling domains and is secreted into the maternal circulation by syncytiotrophoblast cells (Nagamatsu, Fujii et al. 2004). sFlt-1 antagonises VEGF and PlGF by binding to their free circulating forms and thus preventing them from interacting with their receptors (Kendall and Thomas 1993).

VEGF is needed for glomerular capillary repair and thus high levels are expressed by glomerular podocytes (Maharaj, Saint-Geniez et al. 2006). In addition, VEGF receptors can be found on glomerular endothelial cells. Deletion of the podocyte specific VEGF gene in mice results in glomerular endothelial damage and subsequent proteinuria and hypertension (Eremina, Sood et al. 2003). Anti VEGF antibodies have been infused in patients participating in antiangiogenic cancer trials. Again, these patients exhibited hypertension, proteinuria and glomerular endothelial dysfunction (Eremina, Jefferson et al. 2008; Patel, Morgan et al. 2008).

PlGF is a potent angiogenic growth factor, which increases VEGF signalling by displacing VEGF from Flt-1, causing it to bind to the more active VEGFR-2 (Kendall, Wang et al. 1996). PlGF increases angiogenesis in the presence of inflammation, ischaemia and during wound healing (Carmeliet, Moons et al. 2001; Autiero, Lutun et al. 2003). Abnormally low levels of PlGF have been demonstrated in women who develop PET, prior to the onset of clinical symptoms (Levine, Maynard et al. 2004). sFlt-1 decreases cytotrophoblast invasiveness in vitro and is found in low concentration during the first and second trimesters of normal pregnancy (Zhou, McMaster et al. 2002). sFlt-1 levels increase in the third trimester which may be a reflection of the completion of angiogenic placental growth (Levine, Maynard et al. 2004). In vivo, sFlt-1 causes endothelial dysfunction and vasoconstriction and when infused into pregnant rats causes proteinuria, hypertension and glomerular endothelial dysfunction (Maynard, Min et al. 2003). Chorionic villous sample biopsies of women who subsequently developed PET demonstrate altered angiogenic factors including an increased amount of sFlt-1 (Farina, Sekizawa et al. 2008). Not only is increased placental sFlt-1 seen prior to the onset of symptoms, levels are proportional to the severity of disease (Hertig, Berkane et al. 2004; Levine, Maynard et al. 2004; McKeeman, Ardill et al. 2004; Wathen, Tuutti et al. 2006).

Levels of transforming growth factor β (TGF β) and its receptor endoglin, are inversely proportional to cytotrophoblastic invasiveness. In normal pregnancy, levels of TGF β decrease after 9 weeks gestation. However, this decrease is not observed in pre-eclamptic women (Caniggia, Taylor et al. 1997), who also have higher circulating levels of sEng (Venkatesha, Toporsian et al. 2006). sEng is a truncated form of endoglin and also acts as a TGF β receptor and is thought to act by inhibition of

vasodilatation by nitric oxide (NO) (Levine, Lam et al. 2006). Infusion of sEng into pregnant rats amplifies the vascular damage caused by increased sFlt-1 causing them to exhibit a syndrome similar to HELLP (Venkatesha, Toporsian et al. 2006). Interestingly, increased levels of sEng have also been found in small for gestational age pregnancies with no evidence of PET (Levine, Lam et al. 2006; Romero, Nien et al. 2008).

NO is a potent vasodilator and a downstream mediator of VEGF and PlGF. Reduced levels of NO metabolites are seen in women with PET and the amount of NO inhibition correlates with sFlt-1 and sEng levels (Sandrim, Palei et al. 2008).

The existing evidence of alterations in the angiogenic axis in women with PET supports the theory that they have a role in the disruption of cytotrophoblastic invasion which is characteristic of the syndrome.

2.5.6.4 Placental ischaemia and hypoxia

Although placental hypoxia causes increased levels of antiangiogenic proteins, it remains to be seen if PET is a consequence or a result of placental ischaemia and hypoxia. Hypoxia has been shown to increase levels of sFlt-1 in first trimester placental trophoblast cultures (Karumanchi and Bdolah 2004). Previous animal studies have demonstrated that hypertension and proteinuria can be produced by limiting uterine artery blood flow but not HELLP or eclampsia (Granger, LaMarca et al. 2006; Makris, Thornton et al. 2007). Furthermore, placental insufficiency frequently leads to intrauterine growth restriction unaccompanied by the syndrome of PET.

2.5.6.5 Maternal endothelial dysfunction

The diseased placenta releases vasopressive factors into the maternal circulation of pre-eclamptic women (Redman, Sacks et al. 1999). Serum markers of endothelial dysfunction and activation including soluble tissue factor, von Willebrand factor antigen, fibronectin, platelet derived growth factor and endothelin are abnormally elevated in PET (Roberts and Lain 2002). When normal endothelial cells are infused with the serum of pre-eclamptic women, endothelial cell dysfunction occurs which subsequently cause the manifestation of symptoms (Rodgers, Taylor et al. 1988; Roberts, Taylor et al. 1989).

2.5.6.6 Haemodynamic changes:

Normal pregnancy is associated with a physiological decrease in peripheral vascular resistance and hence arterial blood pressure (Roberts and Gammill 2005). In PET, women have a heightened sensitivity to circulating vasopressors such as angiotensin II (Gant, Chand et al. 1974). This, in combination with decreased levels of vasodilators and impaired endothelium-dependant vasorelaxation (Khan, Belch et al. 2005), results in increased systemic vascular resistance and a decreased cardiac output (Easterling 1992). Together, these changes produce the symptom of hypertension.

Angiotensin II hypersensitivity may be a result of autoantibodies which act as agonists for its receptors (Wallukat, Homuth et al. 1999). These same receptor autoantibodies have been shown to impair trophoblastic invasion (Xia, Wen et al. 2003) and when recovered from the serum of women with PET and infused into mice, cause increased secretion and expression of sFlt-1, placental damage and PET symptoms (Zhou, Zhang et al. 2008).

2.5.6.7 Immune changes:

To allow the fetus to develop, the uterus becomes an immune privileged site. Abnormalities in the immune system may contribute to decreased trophoblastic invasiveness. Women with untreated HIV are less likely to develop PET than the general obstetric population and their risk increases to normal levels after antiretroviral treatment (Mattar, Amed et al. 2004). Furthermore, women who conceive via intracytoplasmic sperm injection with sperm collected via testicular biopsy are three times more likely to develop PET than if the sperm is acquired via ejaculation (Wang, Knottnerus et al. 2002). Women who have had decreased contact to sperm due to the routine use of barrier contraception also have a higher PET risk (Klonoff-Cohen, Savitz et al. 1989). When coupled with the fact that PET is more prevalent in nuliparous women, those with new partners and after a long interval between pregnancies, it suggests that an abnormal maternal immune response to fetal antigens may have a role in the pathology of PET.

In addition to the decidual cells which form the bulk of the gravid endometrium, natural killer (NK) cells, macrophages and dendritic cells can also be detected. Together they are the major antigen presenting cells of the uterus and act to prevent the rejection of fetal cells (Huang, Chen et al. 2008). Significantly increased numbers of macrophages and dendritic cells are found in pre-eclamptic placentas (Lockwood, Matta et al. 2006; Huang, Chen et al. 2008). This excess of immune cells stimulates the secretion of tissue necrosis factor- α which induces apoptosis of the extravillous cytotrophoblast (Genbacev, DiFederico et al. 1999).

2.5.6.8 Liver, kidney and cerebral changes:

Pathological changes in these organs are consistent with widespread hypoperfusion. The liver and adrenals both exhibit signs of intraparenchymal haemorrhage, infarction and necrosis. Characteristic changes within the glomeruli are known as glomerular endotheliosis. They include loss of capillary space, vacuolisation of the endothelium and generalised swelling (Spargo, Mc et al. 1959). Subendothelial fibrin deposits decrease the surface area of the glomeruli that can be used for filtration (Lafayette, Druzin et al. 1998). Overall there is a 40% decrease in glomerular filtration rates (Lafayette, Druzin et al. 1998).

Post-mortem findings in eclampsia sufferers include cerebral oedema and intraparenchymal haemorrhage. The oedema is thought to result from endothelial dysfunction as opposed to as a direct consequence of hypertension because the amount of oedema is not proportional to the increase in BP.

Although our understanding of the pathology of PET is continually advancing, the disease remains a leading cause of morbidity and mortality, with implications for both future pregnancies and maternal cardiovascular health. PET frequently presents atypically, mimicking other conditions, resulting in women not being treated appropriately as their symptoms do not meet defined clinical criteria. Screening and diagnostic tests would enable the early identification, enabling clinicians to improve the monitoring and supportive care they provide.

2.5.7 Current Screening methods for PET:

The accurate identification of women at risk of developing PET would enable the implementation of interventions and intensive monitoring to prevent disease progression. Unfortunately the quest for a “perfect screening method” remains elusive.

The following section details the various screening modalities and markers that are in use or being researched at present.

2.5.7.1 Maternal history:

Although risk factors revealed by maternal history only predict severe PET in 30% of cases, NICE recommends its use for screening ((NICE) 2011). The Screening for Pregnancy Endpoints (SCOPE) study tried to stratify healthy nulliparous women into those at high or low risk of developing PET. The study developed a referral framework based on clinical risk factors including: age; blood pressure; BMI; family history of PET or ischaemic heart disease; maternal birth weight; the presence of antenatal vaginal bleeding and notching of increased resistance index on uterine artery dopplers (North RA 2011). However, the authors found that the model was only moderately predictive and recommended the validation of further models (North RA 2011).

2.5.7.2 Blood pressure:

Cnossen et al suggested that a mean arterial greater than 90mmHg in the second trimester is predictive of the onset of PET in a low risk woman with a positive likelihood ratio of 3.5 and a negative likelihood ratio of 0.39. The authors also examined the use of systolic and /or diastolic blood pressure to predict PET. However, both were poor predictors for the disease (Cnossen JS 2008).

2.5.7.3 Uterine artery Doppler

Outside of pregnancy, uterine artery Doppler waveforms consist of a rapid increase followed by a fall of the uterine artery velocity during systole and the narrow calibre high resistance spiral arteries cause notching of the velocity in early diastole. The

second wave of trophoblastic invasion of the spiral arteries converts them into low resistance vessels to facilitate flow through the placenta. This leads to the loss of diastolic notching of the uterine arteries during pregnancy.

Due to impaired trophoblastic invasion of the spiral arteries in PET, the uterine arteries remain in a high resistance state. The placental dysfunction manifests as notching of the uterine artery during diastole and a raised resistance index (RI) and Pulsatility index. RI is a marker for resistance to blood flow in the vascular bed and the PI represents average blood flow (Nicolaidis K 2000). Severe PET in low risk women is best predicted by bilateral uterine artery notching and a raised PI (Cnossen JS 2008). In high risk patients, PET is best predicted using unilateral notching and a raised PI (Cnossen JS 2008). Unfortunately, uterine artery dopplers have a high false positive rate in the prediction of PET so it is currently recommended that they are used in conjunction with further monitoring ((NICE) 2011).

2.5.7.4 Biomarkers

Angiogenic Factors:

Placental ischaemia and the subsequent release of various angiogenic factors is one of the proposed mechanisms of PET. The alterations in the balance of pro and anti-angiogenic factors can be detected in the maternal circulation from the first trimester (Myatt L 2012). Due to elevated sFLT-1 levels and decreased PlGF and VEGF in the circulation of affected women, it has been suggested that there is a higher placental expression of sFLT-1 in PET (Chen 2009). Increased levels of sFLT-1 are seen in pre-eclamptic women from the second trimester onwards (Monte 2011) and lower levels of VEGF and PlGF are evident as early as the first trimester (Romero R 2008).

sEng is increased in the serum and placentas of women with PET, leading to the conclusion that sEng makes a contribution to the pathophysiology of the disease (Hladunewich M 2007). Indeed, altered levels of sEng, sFLT-1 and PlGF levels antecede the clinical manifestations of the disease by several weeks (Chen 2009). A systematic review of 32 studies investigated angiogenic factors' potential to act as biomarkers to predict pre-eclampsia (Kleinrouweler C 2012). Although plasma concentrations of PlGF, sFLT-1 and sEng concentrations were significantly altered prior to 30 weeks gestation in women who developed PET, the accuracies of the four markers were not yet robust for use in clinical practice (Kleinrouweler C 2012).

Uric Acid

Plasma uric acid levels were linked to PET as early as 1917 (Siemons JM 1917). Uric acid is produced during purine breakdown. As gestation increases advances, there is increased cell turnover in the placental bed and hence uric acid concentrations rise progressively. It has been suggested that uric acid concentrations are further raised in PET due higher rates of cell breakdown in the injured placental bed. A systematic review demonstrated that uric acid is an unreliable predictor of PET (Thangaratinam S 2006).

Combination models

As yet no single, reliable biomarker for PET has been identified. Alberry et al explored the possibility of a combination of ultrasound and biomarkers to predict PET (Alberry M 2011). Fetal nucleic acids circulate freely in the maternal serum as free fetal DNA and mRNA and higher circulating levels have been demonstrated in women with PET (Alberry M 2011). Unlike free fetal DNA, mRNA has the advantage of being a sex

independent marker and could have a role in screening either alone or in combination with uterine artery Dopplers.

2.5.8 Prevention of PET:

There is no cure for PET at present. When a woman develops the disease, clinicians must make a decision based on the risk-benefit ratio of prolonging the pregnancy to improve fetal maturity versus the risk of maternal morbidity with continuing the pregnancy. Various measures have been trialled in order to prevent PET before the clinical syndrome develops.

Aspirin:

In PET there is reduced production of prostacyclin, a potent vasodilator and increased quantities of the vasoconstrictor thromboxane, which potentiates platelet aggregation (Briceno-Perez C 2009). Aspirin is a cyclooxygenase (COX) inhibitor and prevents COX from acting as a catalyst in the conversion of arachidonic acid to thromboxane. This limits platelet aggregation in the placental bed. A Cochrane review of 59 trials demonstrated a 17% reduction in PET. Women stratified as high risk for developing PET had a higher risk reduction compared to women at moderate risk (Duley, Henderson-Smart et al. 2007). NICE advice that low dose aspirin be given to women at high risk of PET from 12 weeks gestation until delivery ((NICE) 2011). NICE define women at high risk as the following: those who suffer from chronic hypertension or have previously suffered gestational hypertension in their last pregnancy; those with chronic renal disease or those with autoimmune conditions such as Antiphospholipid syndrome, systemic Lupus erythematosus or diabetes mellitus.

Heparin:

As previously detailed, thrombosis, infarction and necrosis can be seen in the placentas of women who develop PET. Prior researchers have investigated the use of heparin to prevent PET (Kingdom J 2011). Heparin has several modes of action. Firstly, it promotes the proliferation of cytotrophoblast by facilitating FGFs to enhance mitotic signalling. The enhanced production of cytotrophoblast maintains a healthy outer syncytiotrophoblast which interfaces with maternal blood. Secondly, heparin suppresses complement activation, a feature which is enhanced in women that develop PET. Despite these actions, heparin has not been shown to cause a significant reduction in the development of PET (Kingdom J 2011).

Vitamin D

Vitamin D is a fat-soluble protein with anti inflammatory and immune modulatory effects. PET has been linked to vitamin D deficiency (Tabesh M 2013). It was postulated that the enhanced inflammatory response and importance of immune modulators could explain the link. A Cochrane review investigated the effect of vitamin D supplementation on pregnancy outcomes (De-Regil 2012) but of the six trials analysed in the review only one had reducing PET as a primary outcome measure. Women who received 1200 IU vitamin D were not less likely to develop PET (De-Regil 2012). Further studies are needed to determine if different dosages of vitamin D have an effect on PET risk and to determine the safety of supplementation.

2.5.9 Haemostasis in Preeclampsia

At present it is not fully understood how reduced placenta perfusion due to failed vascular remodelling progresses to the multi-system syndrome of PET, or why it only occurs in a proportion of women after the initial insult. In addition to the evidence of

multi organ hypoperfusion, previously mentioned evidence of widespread microthrombi deposition is also present in women who die from eclampsia (Roberts and Lain 2002). Abnormalities of haemostasis which result in, or cause excess uteroplacental thrombosis, may contribute to the pathophysiology of PET.

2.5.9.1 Haemostasis in the systemic circulation

Procoagulants:

Women with PET display exaggerated coagulation activation compared to the general obstetric population with coagulation markers including TAT (a surrogate marker of thrombin generation) (Chaiworapongsa, Yoshimatsu et al. 2002), TF (Rousseau, Favier et al. 2009), FVIII consumption (Thornton and Bonnar 1977) and fibrinogen (Bonnar, McNicol et al. 1971; Halligan, Bonnar et al. 1994) showing additional increases. In addition, women with PET demonstrate increased endogenous thrombin potential (ETP- a global assay of haemostasis) when compared with normotensive pregnant controls (Rafik Hamad, Curvers et al. 2009) who in turn have a higher ETP than non-pregnant women (Rosenkranz, Hiden et al. 2008). Interestingly in pre-eclamptic women, enhanced ETP is associated with increased platelet activation which is not present in the normotensive population. This may account for the benefits of treatment with aspirin in PET (a 17% reduction in the risk of developing PET; a 14% reduction in neonatal mortality and 8% lower risk of premature delivery) (Duley, Henderson-Smart et al. 2007).

Anticoagulants:

Contradictory evidence is available regarding the changes in antithrombin levels in normal pregnancy. Several studies have found that antithrombin levels remain unchanged during (Stirling, Woolf et al. 1984; Rosenkranz, Hiden et al. 2008) and

others have demonstrated decreased levels in the third trimester and postpartum period (Wickstrom, Edelstam et al. 2004; Tanjung, Siddik et al. 2005). Significantly decreased levels of AT are seen in women with PET compared with controls (Tanjung, Siddik et al. 2005) which may be a reflection of increased consumption.

An additional key regulator of thrombin generation is tissue factor pathway inhibitor (TFPI). Two types exist: TFPI-1 which is found in the maternal and fetal circulations and TFPI-2 which is mainly found in the placenta. In normal pregnancy, concentrations of TFPI-2 gradually increase with gestation to peak at 36 weeks, after which they decline (Chand, Foster et al. 2005). Women with PET have significantly higher concentrations of both TF and TFPI (Abdel Gader, Al-Mishari et al. 2006; Erez, Romero et al. 2008). However, the TF:TFPI ratio is lower than in the normal population, suggesting that the increase TFPI levels are insufficient to counteract the increase in TF (Erez, Romero et al. 2008).

2.5.9.2 Fibrinolysis in PET

Fibrinolysis is dependent upon the balance of basal levels of tPA, PAI-1 and tPA released from the endothelium as a reaction to vascular injury or thrombus formation (Oliver, Webb et al. 2005). By the third trimester, there is a 4-5 fold increase in PAI-1 in the normal pregnant population (Coolman, de Groot et al. 2006; Robb, Mills et al. 2009). PAI-1 inhibits the release of endothelial tPA, thus the increased levels of PAI-1 in normal pregnancy further reduce tPA levels (Robb, Mills et al. 2009). PAI-2 is produced by the placenta, accordingly levels increase with gestation as the placental mass grows (Reith, Booth et al. 1993).

When affected by PET, women exhibit an additional increase in PAI-1 compared to normotensive pregnant controls which precedes the onset of clinical symptoms and

evidence of placental dysfunction detected as uterine artery Doppler notching (Hunt, Missfelder-Lobos et al. 2009). Contrastingly, PAI-2 levels in PET are significantly lower than in normal pregnancy, most likely due to placental dysfunction (Tanjung, Siddik et al. 2005).

2.5.9.3 Uteroplacental circulation

As previously mentioned, there are three distinct haemostatic challenges in pregnancy: i) implantation, where trophoblast invades the maternal decidual vessels; ii) the antenatal period during which the blood must remain fluid enough to flow readily through the low resistance system of the uteroplacental unit and finally; iii) delivery where coagulation has to occur quickly in order to prevent life threatening haemorrhage from the placental bed. TF appears to be the key procoagulant during implantation. It is produced by maternal human endometrial stromal cells (HESC) (Lockwood, Krikun et al. 2000) which enable the trophoblastic invasion of the decidual vessels to occur without massive haemorrhage occurring. The overall haemostatic balance of implantation is maintained by TF, TFPI-1 produced by endothelial cells and TFPI-2 released from the placental trophoblast (Dusse, Carvalho et al. 2006). This is supported by the findings of animal studies where the TF or TFPI genes are inactivated in mice result in miscarriage (Parry and Mackman 2000; Pedersen, Holscher et al. 2005).

In PET placental TF mRNA expression is increased (Estelles, Gilabert et al. 1998; Dusse, Carvalho et al. 2006), but TFPI mRNA expression and total TFPI concentrations are lower than the general obstetric population (Aharon, Lanir et al. 2005; Dusse, Carvalho et al. 2006). Thus, although both coagulation and fibrinolysis are activated in

the uteroplacental circulations of both normal and pre-eclamptic women, PET is associated with notable abnormalities.

Chapter 3

LABORATORY METHOD

3.0 TAFI Antigen and Activity assays

There are three main methods to determine TAFI plasma concentrations: immunological, activity based and functional assays. When designing a study protocol, it is particularly important to consider the advantages and drawbacks of each method. Many previous studies have used assays that were inadequately validated, making interpretation of their results difficult.

3.1 Immunological assays:

Historically, this has been the most commonly used method of measuring TAFI levels. It has the advantages of: being relatively quick and easy to perform using commercially available kits; the zymogen is measured directly and does not require any preliminary activation and there is no interference from carboxypeptidase N (CPN). Furthermore, as TAFI plasma concentrations are well below the *K_m* for activation by the thrombin-thrombomodulin complex the amount of TAFI_a formed will be dependent upon the TAFI Ag concentration.

However, immunoassays have two major drawbacks. Prior studies have reported a degree of genetic control of TAFI Ag plasma concentrations (Chetaille, Alessi et al. 2000; Henry, Aubert et al. 2001). A single nucleotide polymorphism (SNP) at position 1040 results in the substitution of Thr-to-Ile at position 325 in the protein. The Ile325 TAFI variant has a much longer half life than the Thr325 variant (15 minutes compared to 8-10 minutes) (Boffa, Bell et al. 2000; Schneider, Boffa et al. 2002). The immunoreactivity of the TAFI isoform is strongly affected by this SNP and its resultant substitution (Gils, Alessi et al. 2003). Gils et al optimised two ELISAs: the first was not affected by the 325 genotype but the second demonstrated no reaction with the Ile325 genotype (Gils, Alessi et al. 2003). One commercially available ELISA kit,

which uses two monoclonal antibodies, has been shown to be free of genotype-based bias. The various commercially available ELISA kits yield notably different results. Thus, accurate comparison of data generated by different studies will not be possible until an international standard for TAFI Ag is defined.

Secondly, although plasma TAFI is predominantly in the form of TAFI Ag, it also exists in various other states including TAFI Ag bound to plasminogen and inactive TAFI fragments. The antibodies in the ELISA kit can have different reactivities to these various forms (Gils, Alessi et al. 2003). Again, this can produce different results for the same sample when different monoclonal antibodies are used in the ELISA kits.

Normal pooled plasma is used to calibrate the ELISA assays. The dilutional profile of the pooled plasma can also be affected by genotype frequencies, i.e. it can be more similar to Thr325 than Ile325 genotype pooled plasma or vice versa. This can produce further discrepancies in the ELISA results which may account for the wide variation of the reported mean plasma TAFI Ag concentrations (Guimaraes, van Tilburg et al. 2004). This further emphasises the necessity of meticulous standardisation of the assays used in further studies.

Other immunological assays including Rocket immunoelectrophoresis have been reported. This method has the advantage of being insensitive to genotype bias. However, the method is laborious, making it impractical to use in large studies such as ours.

3.2 Enzymological assays:

These assays are based on the principal that when activated TAFIa can cleave the C-terminal lysine or arginine from various protein and peptide substrates upon the addition of thrombin/ thrombomodulin to the plasma sample. Hippuryl-arginine/ lysine is a peptide substrate that is converted to hippuric acid by TAFIa. This conversion can be monitored by measuring the change in the absorbance.

The main benefit of enzymological assays is that only the functionally active TAFIa is quantified and the various fragments of TAFI Ag that have undergone proteolytic cleavage or conformational alterations are not included. Furthermore, both the Thr325 and Ile325 isoforms of TAFI Ag have comparable activation kinetics and once activated both have similar carboxypeptidase activity toward the synthetic substrates used in the assay (Zhao, Morser et al. 1998; Schneider, Boffa et al. 2002). Due to the extra step of activation of TAFI Ag required, this type of assay is more time consuming than immunoassays and requires a robustly standardised activation protocol (Bajzar, Morser et al. 1996; Schatteman, Goossens et al. 2000). Furthermore, in some commercially available assay kits, the substrates used are not selective for TAFI. Therefore, a small proportion of the lysine/ arginine residues produced will be a result of the intrinsic activity of other carboxypeptidases including CPN.

A further drawback in activity-based assay is that they are affected by the intrinsic instability of TAFIa and its different isoforms. Placing the samples on ice can help stabilise TAFIa after activation by arresting the conformational change that renders the protein inactive (Schneider, Boffa et al. 2002). Excess substrate can also stabilise TAFIa (Willemsse and Hendriks 2006). However, if due to the amount of TAFIa produced, all the substrate is converted, the TAFIa will begin to decay and thus TAFI

activity underestimated. Thus, large quantities of substrate are added to overcome this difficulty.

3.3 Functional Assays

Global assays of haemostasis evaluate the net coagulation/ fibrinolytic capacity. Although they are unable to detect specific haemostatic abnormalities, they are often of more practical value when assessing thrombotic/-bleeding risk in clinical practice. Advances in the continuous measurement of thrombin generation in plasma have been used in clinical coagulation research to further the understanding of coagulation in neonates (Cvirn, Gallistl et al. 1999; Cvirn, Gallistl et al. 2003). However, thrombin generation cannot be used to assess fibrinolysis. Many global assays of haemostasis have been developed, but the two most frequently used in clinical practice are the thromboelastogram and euglobin clot lysis time (CLT).

CLT measures plasma fibrinolytic activity and was originally described in 1948 (Macfarlane and Biggs 1948). It measures changes in optical density of the recalcified euglobulin fraction of plasma over a two hour period. Prior studies have demonstrated that CLT is not prolonged by short term storage of frozen samples (Prisco, Paniccia et al. 1994).

Guimaraes et al described an assays designed to assess the total amount of functional TAFI in plasma samples based on clot lysis times (Guimaraes, van Tilburg et al. 2004). Immunodepletion is used to produce TAFI deficient plasma which is then added to the plasma sample undergoing testing. The mixture is then allowed to clot in the presence of thrombomodulin which activates TAFI. Subsequent clot lysis is then quantified by measuring the turbidity of the mixture in a microplate reader. Duplicates of each sample are run simultaneously, to which potato tuber carboxypeptidase inhibitor

(PTCI) (a TAFI specific inhibitor) have been added. This allows the quantification of the contribution of TAFIa to the retardation of clot lysis. This assay is sensitive to plasma antigen levels and to which isoform is present (Guimaraes, van Tilburg et al. 2004).

To overcome to limitations of each of the above methods, in the following studies all three assays were performed for each of the samples collected. This enabled quantification of TAFI zymogen levels, TAFI's activity and its contribution to global haemostasis.

3.4 Methods used in this thesis:

3.4.1 TAFI Activity (TAFI Actichrome®)

The Actichrome kit (American Diagnostica) is a commercially available chromogenic assay used to determine the concentration of activateable TAFI in plasma samples. The assay is dependent upon the activation of TAFI by the thrombin-thrombomodulin complex. The TAFIa that forms then reacts with a chromogenic substrate which is highly specific for TAFIa.

The initial step of the assay is to incubate plasma samples (which have been diluted 25-fold in the assay buffer) with the TAFI activation reagent in a microtitre plate well. The thrombin-thrombomodulin complex in the activation reagent converts TAFI into TAFIa. An Activation Stop reagent is then added to the plate to arrest TAFI activation. Finally the TAFI Developer, containing the TAFI specific substrate is added to the well and an enzymatic reaction ensues. This reaction is then terminated by the addition of sulphuric acid so the optical density of the solution at 405nm can be quantified. A plain diluted plasma sample which has not been incubated with the TAFI activation reagent is run in parallel as a control. The difference in optical density between the activated

plasma sample and the control, representing the amount of TAFI activity in the sample, can then be calculated.

Using the TAFI standard included in the kit, a calibration curve was created. The absorbances of the samples were then compared to the calibration curve.

3.4.2 TAFI Antigen Concentration (ELISA)

TAFI Ag was quantified using a commercially available kit (Zymutest TAFI Ag, Hyphen Biomedical) for a sandwich ELISA specific for human TAFI Ag. The pre diluted plasma samples were placed into microwells pre-coated with a monoclonal antibody specific for human TAFI. The TAFI Ag present in the plasma sample binds to the monoclonal antibody. The wells are then washed and the immunoconjugate added to the plasma samples in the wells. The immunoconjugate consists of a goat polyclonal antibody coupled with horse radish peroxidase. It reacts with the TAFI Ag in the wells. After the wells have again been washed, the peroxidase substrate (tetramethylbenzidine (TMB) and hydrogen peroxide) is added to the wells which turn blue. Finally, the reaction is stopped with sulphuric acid and the wells turn yellow. The colour density is proportional to the plasma concentration of TAFI Ag.

3.4.3 Clot Lysis Time:

This is a functional assay of TAFI, which works on the principal that TAFIa will retard clot lysis mediated by tPA in a plasma sample. Test plasma samples are mixed with reagents listed in table 1 and is then left to incubate, so clot formation can occur in conjunction with TAFI activation by thrombomodulin. Subsequent clot lysis as a result

of TAFI activation is quantified using a microplate reader which measures changes in turbidity. A control assay (using PTCI to specifically inhibit TAFIa) is run in parallel. This allows for the quantification of the contribution of TAFI to the retardation of clot lysis.

Table 4: Materials required for TAFI antigen and activity levels and Clot lysis time analysis

	TAFI Activity	TAFI Antigen	Clot Lysis Time
Materials required	<ul style="list-style-type: none"> • 1x96 well microtest plate • 1 vial TAFIa Standard • 20ml assay buffer • 1 vial TAFI Developer • 1 vial TAFI Activation Reagent • 1 vial TAFI Stop Reagent • 1 vial Activation enhancer • 0.22 µm filtered deionised water • 8 channel pipette (50-200µL) • 1 channel pipette (10-200µL) • 1.5 ml microfuge tubes • Microwell plate orbital shaker • Microwell plate reader set at a wavelength of 490nm • 2M Sulphuric acid 	<ul style="list-style-type: none"> • Micro ELISA plate • 100ml F-Sample diluent • 3 vials of Plasma TAFI calibrator (normal plasma calibrated with a reference pool) • 1 vial TAFI Control I • 1 vial of TAFI Control II • 3 vials Anti-(H)-TAFI-HRP immunoconjugate • 25ml conjugate diluent • 50 ml washing solution • 25ml 3,3',5,5' Tetramethylbenzidine • 0.45M Sulphuric acid • 8 channel pipette (50-300µL) • 1 channel pipette (0-1000µL) • Micro ELISA washing equipment and shaker • Micro ELISA plate reader (set up to read at 450 nm) 	<ul style="list-style-type: none"> • Innovin (0.6 picomM) • 1M Calcium chloride • Phospholipid buffer • tPA [1680ng/ml] • Potato tuber caboxypeptidase inhibitor (PTCI)[25µg/ml] • HEPES Buffer (25mM, 2.98g/500ml) • 20% BSA • Flat bottomed NUNC plates
Expected range	12-20µg/ml	40-250%	

There is a great deal of heterogeneity amongst the current literature, with some studies reporting TAFI antigen and others TAFI activity and CLT. This has led to conflicting data being reported. In order to gain a broader perspective of changes in TAFI during pregnancy all three methods were used in the following studies. Not only does this allow comparison with available published data, it enables investigators to determine if changes in TAFI activity differ from those of antigen levels. This may contribute to the understanding of various pregnancy related pathology including PET.

Chapter 4

Changes in TAFI in uncomplicated pregnancy

4.1 INTRODUCTION:

There are marked changes in coagulation parameters in normal pregnancy (Franchini 2006). Many pro-coagulant factors increase during pregnancy creating a pro-thrombotic state (Kadir R 2009; Huq FY 2011). This is most likely a physiological attempt to limit bleeding after delivery. Szecsi et al recently established reference ranges for coagulation factors in pregnancy and the puerperium (Szecsi PB 2010). They reported: significantly increased levels of fibrinogen Factors VII, VIII, and IX; unchanged levels of factors II, V, X, XI, XII, protein C and antithrombin and decreased levels of protein S (Szecsi PB 2010). However, TAFI was not included in the study (Szecsi PB 2010).

Changes in TAFI levels during pregnancy have previously been studied. However, reports in the literature are contradictory (Chabloz, Reber et al. 2001; Mousa, Downey et al. 2004; Watanabe, Minakami et al. 2004).

This study aims to determine the nature of changes in TAFI in normal pregnancy and create pregnancy specific reference intervals TAFI Ag and TAFIa.

4.2 MATERIALS AND METHODS:

4.2.1 Study design:

This was a prospective longitudinal study of 130 women who attended the Obstetric units of two hospitals (Royal Free Hospital and Colchester General Hospital). Written informed consent to participate in the study was obtained from all subjects. Women were recruited from antenatal clinic. The study protocol was reviewed and approved by the Royal Free Hospital Ethics committee (see appendix 1).

4.2.2 Sample size:

The sample numbers were chosen in line with the International Federation of Clinical Chemistry's (IFCC) recommendation that a minimum sample size of 120 participants be used to calculate the reference range for a parameter (Institute 2008).

4.2.3 Inclusion and Exclusion criteria:

Only women with an uncomplicated singleton pregnancy were approached. Women with a known history of: thrombophilia; thromboembolic disease; systemic lupus erythematosus; recurrent miscarriage; antiphospholipid syndrome; diabetes mellitus; hypertension; acute or chronic renal failure or other significant medical co-morbidity were excluded from the study. The participants' maternity records were reviewed after their deliveries. Any participant who developed a complication (e.g. a hypertensive disorder of pregnancy, IUGR, venous thrombotic disease or pre-term delivery) during their pregnancy was excluded. None of the participants were receiving non-steroidal anti-inflammatory, anti-platelet or anticoagulant therapy.

4.2.4 Data collection:

The following demographic data was collected prospectively for all study subjects: maternal age; parity; blood pressure and weight at antenatal booking visit (conducted by ten weeks gestation) and smoking history.

Pregnancy gestation was calculated using the dating scan performed between 11-13+6 weeks. This scan is offered to all women receiving antenatal care in the UK, and determines the expected date of delivery using crown rump length. Samples were collected from the participants in each trimester of pregnancy (first trimester samples between 0 and 12 weeks; second trimester between 13 and 28 weeks and the third trimester between 29 and 42 weeks).

4.2.5 Blood collection

Venous whole blood samples were collected for each of the 130 participants in the first, second and third trimesters of pregnancy. Sample collection was performed in the sitting position by direct venepuncture and collected into citrated tubes. Plasma samples were then prepared from citrated whole blood centrifuged at 2500g for 20 minutes, divided into aliquots and frozen at -70° C until analysis. Samples obtained at Colchester General Hospital were centrifuged and divided into aliquots on site and then transported to the Royal Free Hospital Haemophilia Centre laboratory for analysis. TAFI antigen (TAFI Ag), TAFI activity (TAFIa) and clot lysis time (CLT) assays were then performed.

4.2.6 Laboratory Method

The following assays were performed on each collected sample: TAFI Ag levels, TAFIa and CLT in the presence of a buffer solution and CLT with PTCI (a TAI inhibitor). All analysis were performed as described in chapter 3.

4.2.7 Statistics

Descriptive statistics were utilised for demographic data. Continuous data were presented as mean and standard deviation. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software (SPSS Inc. Released 2009. PASW Statistics for windows, Version 18.0. Chicago:SPSS Inc).

In order to determine if the collected data had a normal distribution both visual and statistical methods were employed. A histogram created using SPSS demonstrated that the data was not normally distributed. This was then confirmed using the Shapiro-Wilk test in SPSS. Both methods demonstrated that the data did not have a normal distribution, hence non-parametric tests were used in the analysis. Comparisons between trimesters were performed using the Wilcoxon rank test. Spearman correlation was used for correlation analysis. A p value of <0.05 was accepted as statistically significant.

4.3 RESULTS:

4.3.1 Population and Demographic data:

One hundred and thirty women were recruited to participate in this part of study. Seventy three samples were collected from Colchester General Hospital and fifty seven from the Royal Free Hospital. Three women developed PET and thus excluded. Three second trimester samples were thawed and unsuitable for analysis, and one subject transferred her care to an alternate hospital in her third trimester and was lost to follow up.

Table 5: Number of Samples analysed in each trimester of normal pregnancy

	Number of samples analysed		
	TAFI Ag	TAFIa	CLT
Trimester 1	127	127	104
Trimester 2	124	124	107
Trimester 3	126	126	102

The demographic data for the study participants is presented in table 5. The median age and parity of the participants were 32 (range 18-43) years and 1 (range 0-4) respectively.

Table 6: Distribution of age, parity and booking weight and blood pressure in normal pregnancy

	Participants (n=127)
Parity:	
Unknown	0 (0%)
0	36 (28%)
1	49(39%)
2	38 (30%)
3+	4 (3%)
Smoker	
Unknown	6 (5%)
Yes	7 (6%)
No	114 (89%)
Age (years):	
Median (range)	32 (18-43)
Booking weight (kg)	
Median (range)	66 (48-125)
Systolic Blood Pressure at booking (mmHg)	
Median (range)	111 (94-132)
Diastolic Blood Pressure at Booking (mmHg)	
Median (range)	70 (54-86)

4.3.2 Changes in TAFI Ag, TAFIa and CLT with gestation in normal pregnancy:

The mean, median and reference ranges of TAFI Ag, TAFIa and CLT are determined and presented in table 6a, 7 and 8.

Overall, TAFI Ag, TAFIa and CLT increased with gestation, with the highest levels/ longest times seen in the third trimester. (see Table 6a and 6b).

Table 7a: Mean TAFI Ag, TAFIa and CLT throughout pregnancy

	Mean	Median (range)
TAFI Ag (%) Trimester 1	123.7	109.5 (56-318)
TAFI Ag (%) Trimester 2	143.5	136 (62-280)
TAFI Ag (%) Trimester 3	153.6	153 (56-280)
TAFIa (mcg/ml) Trimester 1	25.5	25 (5.8-53)
TAFIa (mcg/ml) Trimester 2	28	27.5 (10-59)
TAFIa (mcg/ml) Trimester 3	50.7	30 (9-70)
CLT (min) Trimester 1	69.4	68 (39-180)
CLT (min) Trimester 2	87.0	82 (39-240)
CLT (min) Trimester 3	99.6	93 (12-240)

TAFI Ag levels:

Mean TAFI Ag levels increased with gestation with the highest levels seen in the third trimester.

Median TAFI Ag levels progressively increased with gestation with the highest levels seen in the third trimester.

There was a significant increase in mean TAFI Ag levels between the first and second and the first and third trimesters ($p=0.005$ and $p=0.006$ respectively). Levels did not increase significantly between the second and third trimesters ($p=0.190$).

TAFIa levels:

TAFIa mirrored TAFI Ag levels. Mean TAFIa levels increased with gestation with the highest levels seen in the third trimester.

Median TAFIa levels also increased with gestation with the highest levels seen in the third trimester.

There was a statistically significant increase in mean TAFIa between the first and second and the first and third trimesters ($p=0.0005$, $p=0.0005$ respectively). Again, no significant increase was seen between the second and third trimesters ($p=0.125$).

CLT:

CLT in the presence of a buffer solution increased significantly with gestation. Mean CLT became longer with gestation and the longest times were seen in the third trimester.

Median CLT progressively increased with the longest times seen in the third trimester. Significantly longer times were seen between the first and second, first and third and second and third trimesters ($p=0.001$, $p=0.009$ and $p=2.5 \times 10^{-6}$ respectively).

Table 7b: Statistical significance of changes in the mean values of TAFI antigen, activity and clot lysis time between trimesters

Significance of difference between means in:

	Trimesters 1 and 2	Trimesters 1 and 3	Trimesters 2 and 3
TAFI Ag	0.005*	0.006*	0.190
TAFIa	0.0005*	0.0005*	0.125
CLT	0.001*	0.085*	P<0.0001*

**denotes statistical significance where $p < 0.05$*

Figure 4: Changes in Mean TAFI Ag levels throughout pregnancy

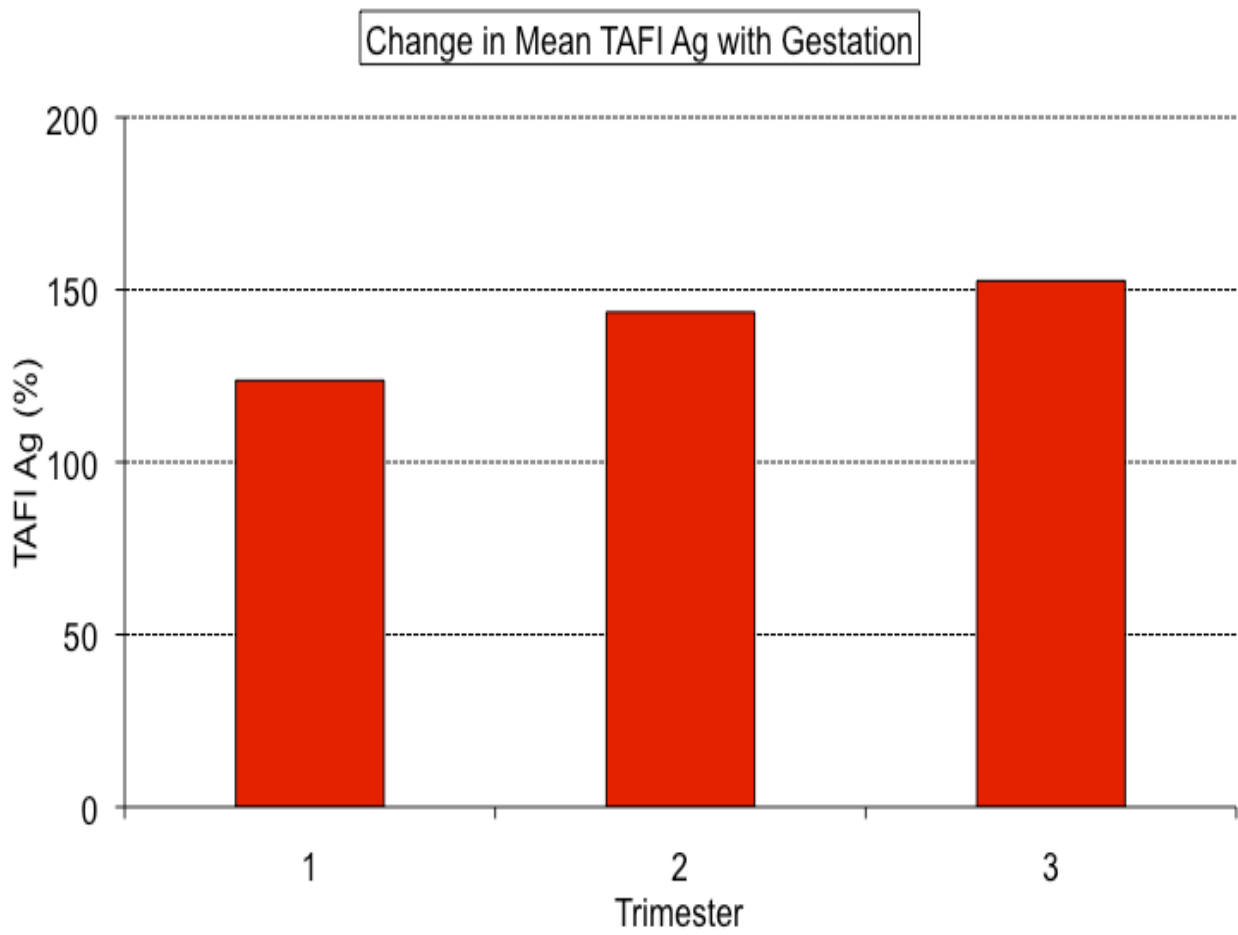


Figure 5 : Changes in Mean TAFIa levels throughout pregnancy

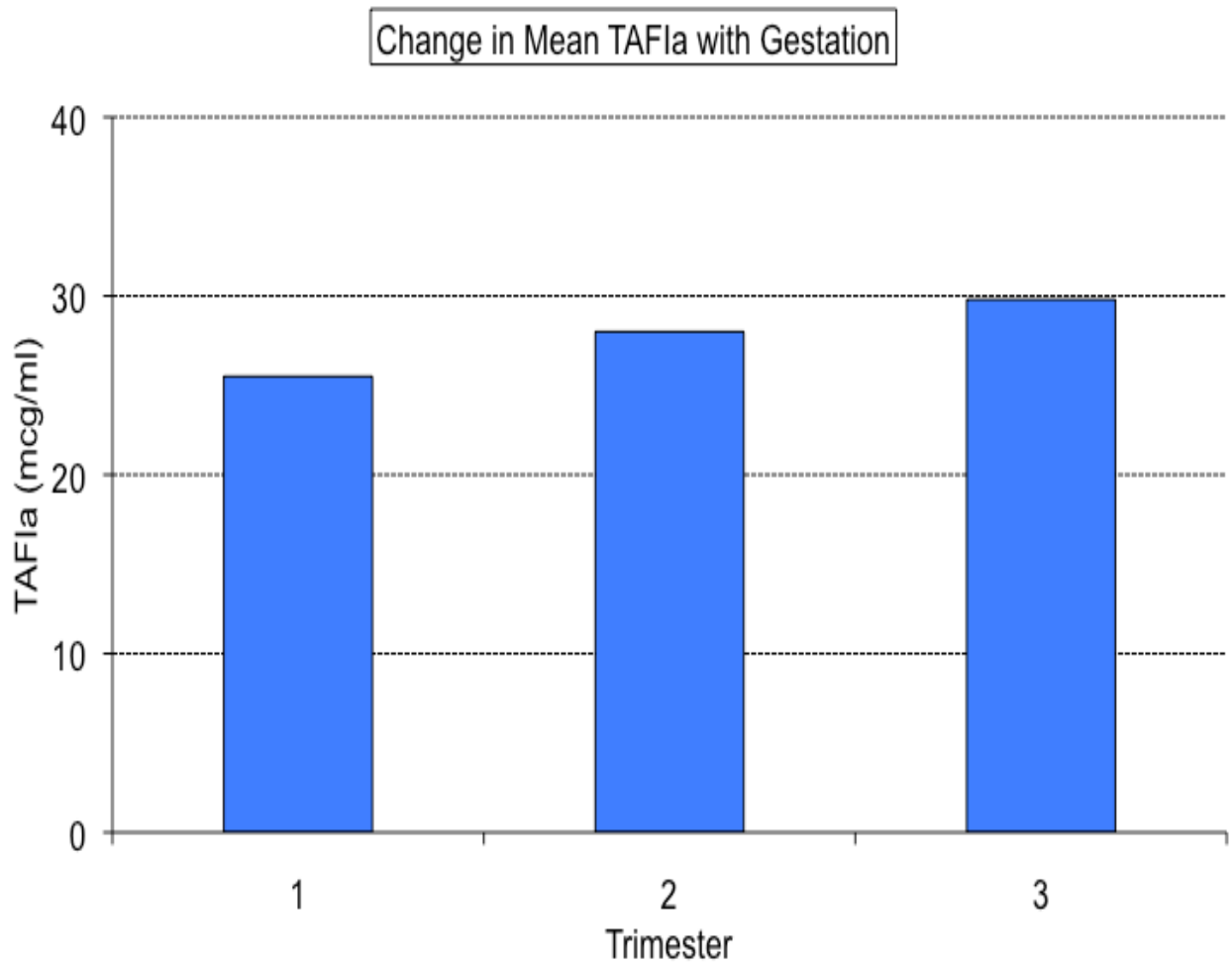


Figure 6: Changes in Mean CLT throughout pregnancy



Figure 7: Changes in TAFI Ag with gestation

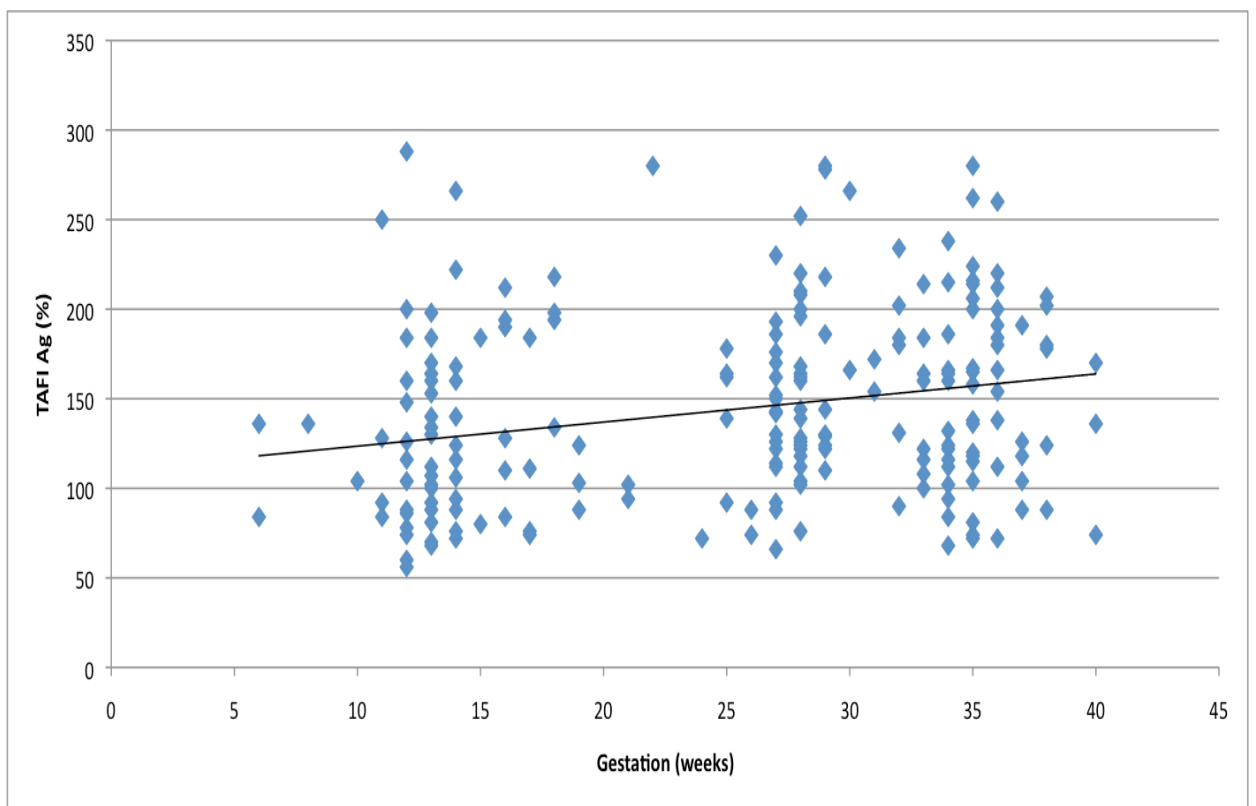
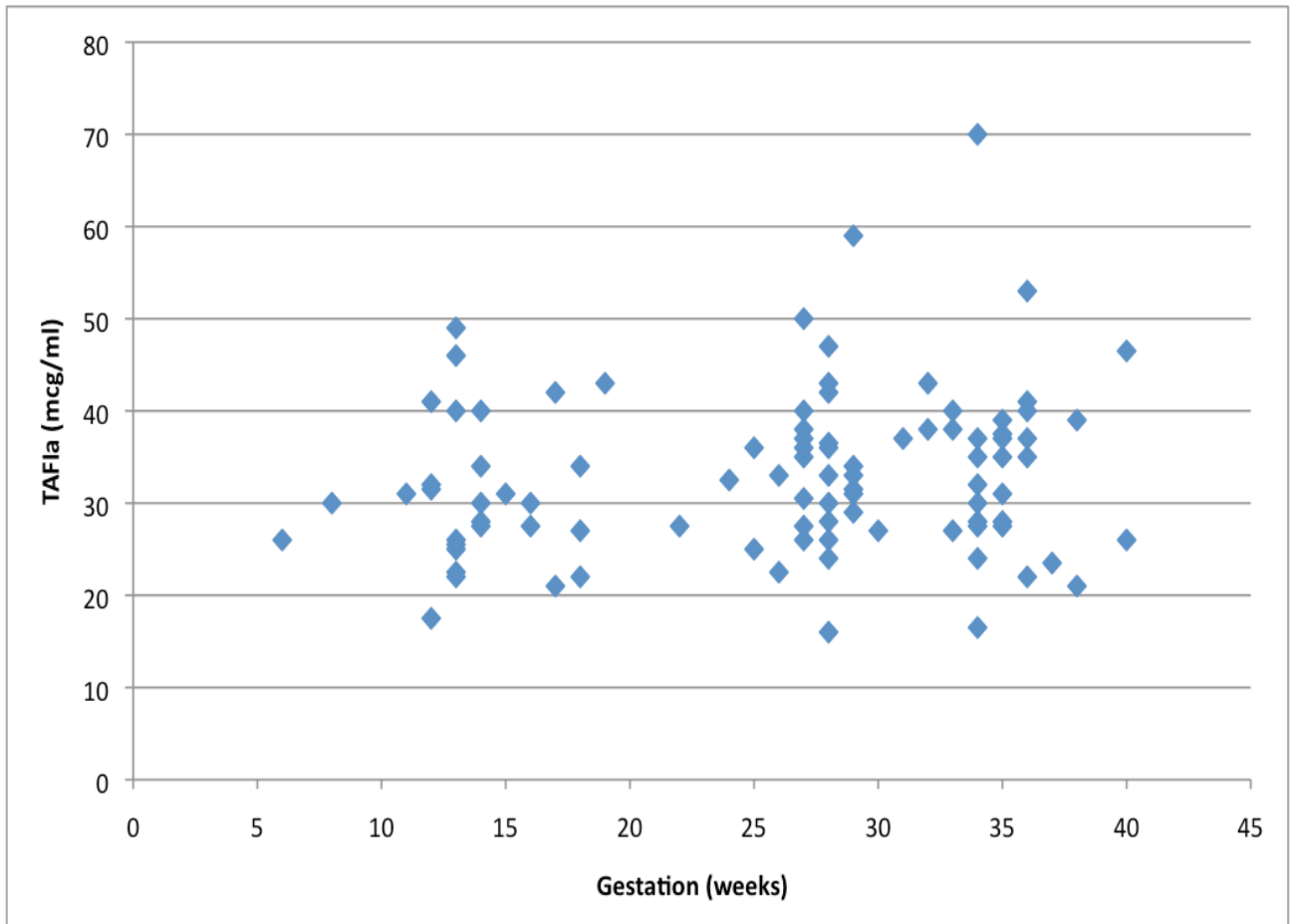


Figure 8: Changes in TAFIa with gestation



4.3.3: Reference ranges for TAFI in normal pregnancy:

The reference intervals for TAFI Ag and TAFI a in each trimester are show in the table below.

Table 8: Reference intervals of TAFI Ag and TAFIa during each trimester of normal pregnancy

Reference Interval	Lower Quartile	Median (range)	Upper Quartile	Mean	Standard deviation
TAFI Ag (%) 1 st Trimester	89.5	109.5 (56-318)	142	123.7	48.7
TAFI Ag (%) 2 nd Trimester	110	136 (62-280)	168	143.5	49.2
TAFI Ag (%) 3 rd Trimester	116	153 (56-280)	186	152.6	50.7
TAFIa (mcg/ml) 1 st Trimester	21	25 (5.8-53)	31	25.5	9.6
TAFIa (mcg/ml) 2 nd Trimester	22.4	27.5 (10-59)	34	28.0	10.1
TAFIa (mcg/ml) 3 rd Trimester	24.5	30 (9-70)	35	50.7	9.4

Table 9: Reference ranges of TAFI Ag and TAFIa in normal pregnancy in each trimester (Values are mean+/- SD (95% confidence intervals))

TAFI Ag (%) 1 st Trimester	123.7 +/- 48.7 (26.2-221.1)
TAFI Ag (%) 2 nd Trimester	143.5 +/- 49.2 (45.2-241.9)
TAFI Ag (%) 3 rd Trimester	152.6 +/- 50.7 (51.3-254.0)
TAFIa (mcg/ml)1 st Trimester	25.5+/- 9.6 (6.4-44.7)
TAFIa (mcg/ml)2 nd Trimester	28.0 +/- 10.1 (7.8-48.3)
TAFIa (mcg/ml) 3 rd Trimester	29.8 +/- 9.4 (10.9-48.6)

Figure 9: Distribution of TAFIa in each trimester of normal pregnancy

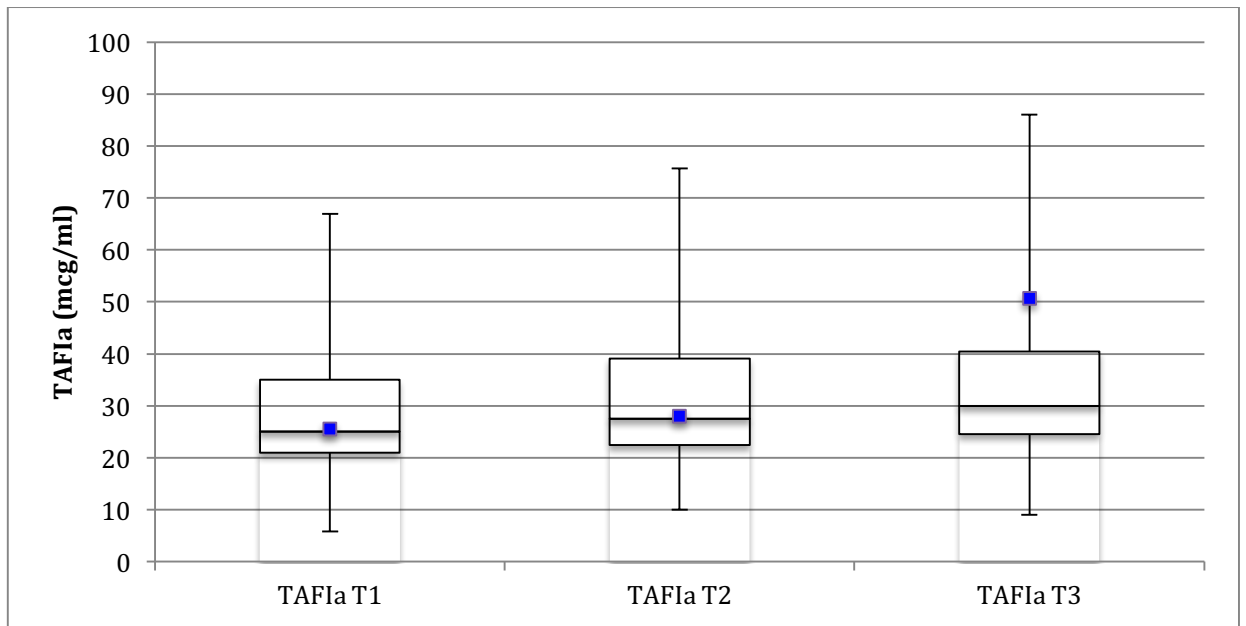


Figure 10: Distribution of TAFI Ag in each trimester of normal pregnancy

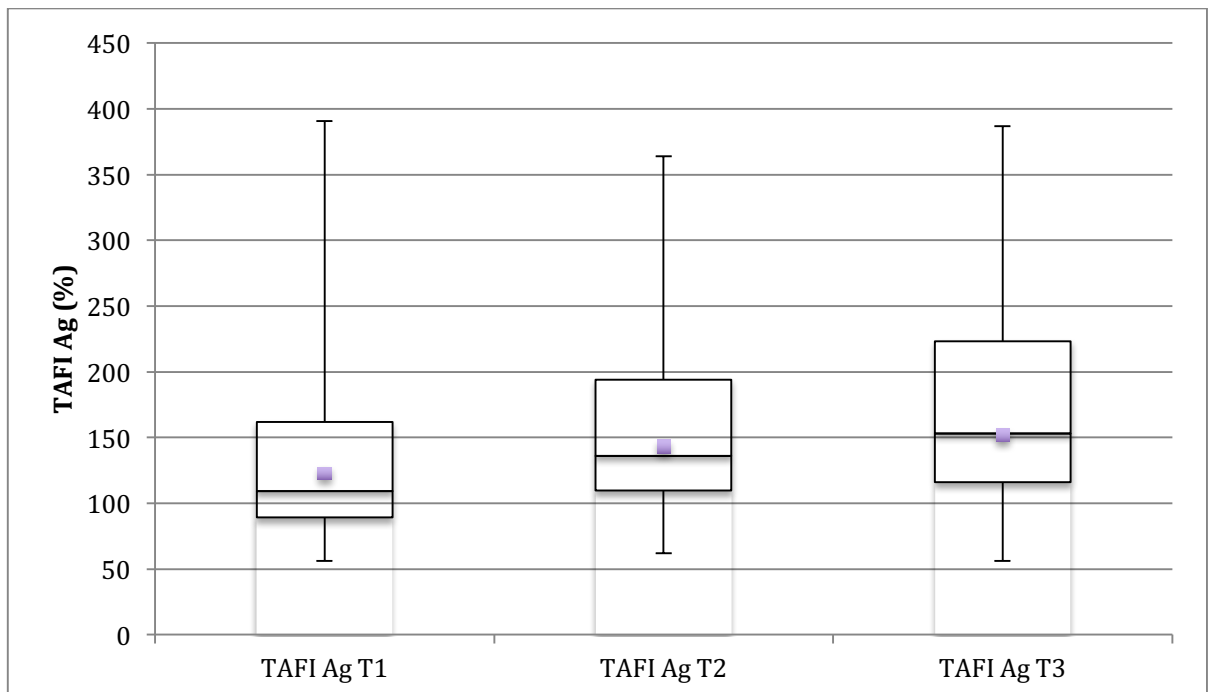


Figure 11: Distribution of CLT in each trimester of normal pregnancy

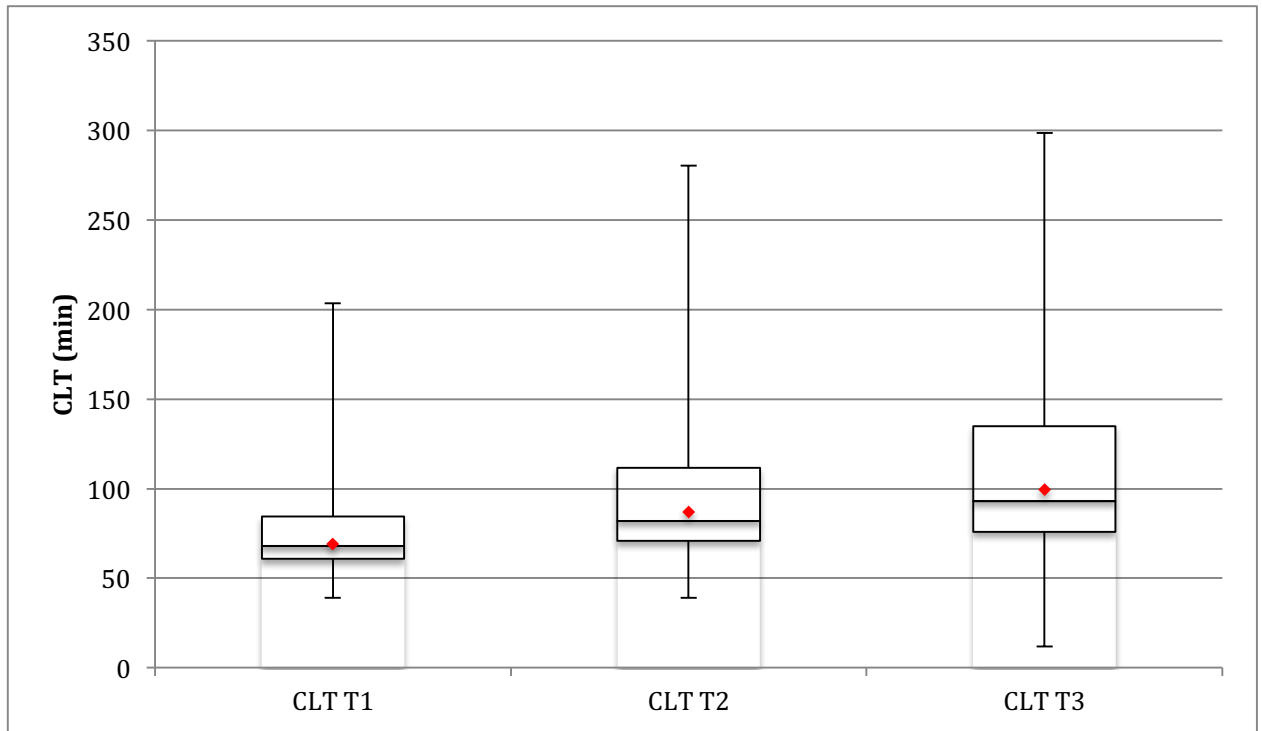


Figure 12: The distribution of TAFIa in the first trimester

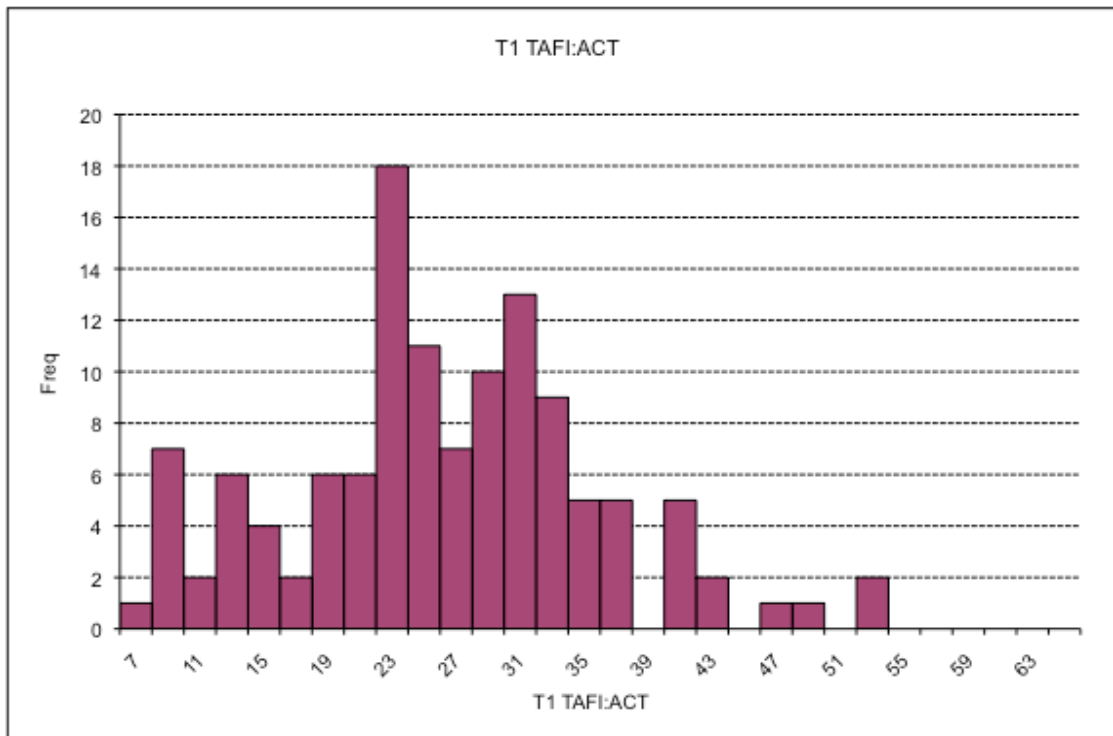


Figure 13: The distribution of TAFIa in the second trimester

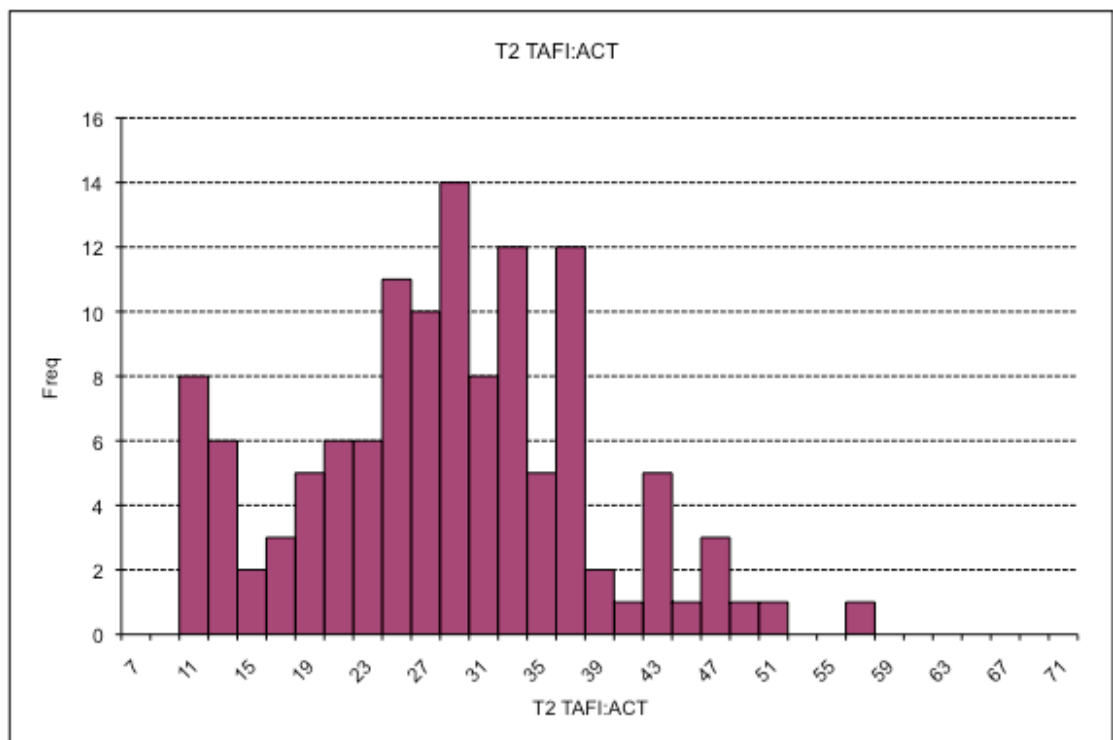


Figure 14: The distribution of TAFIa in the third trimester

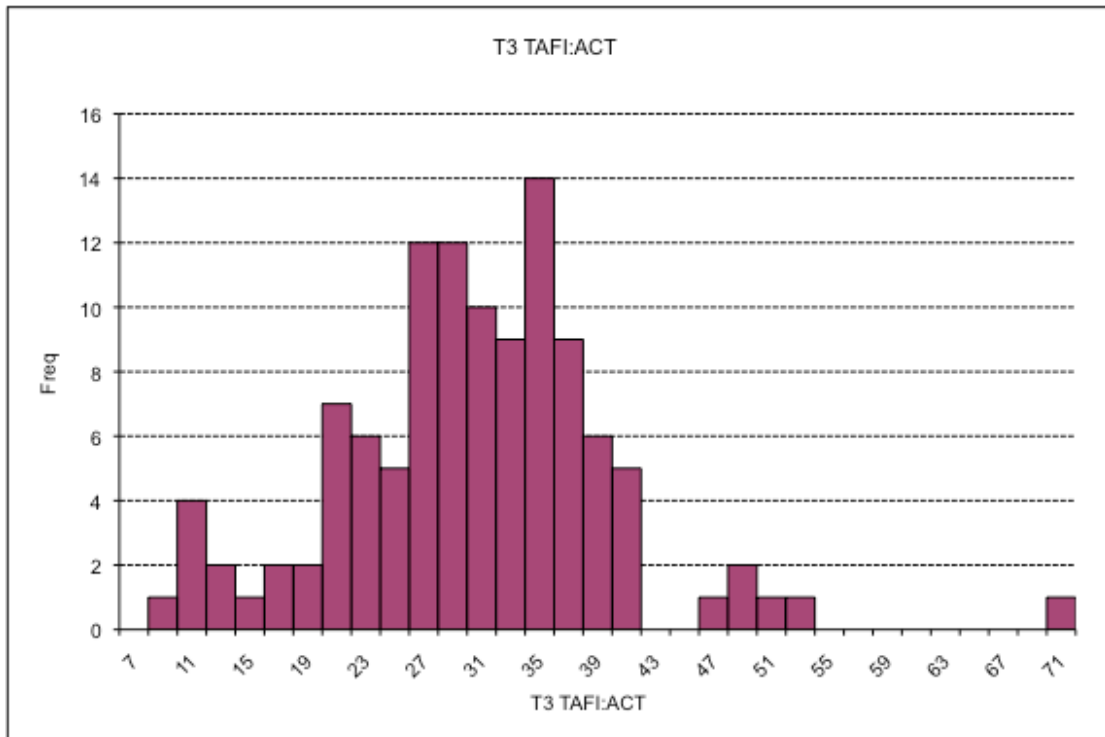


Figure 15: The distribution of TAFI Ag in the first trimester

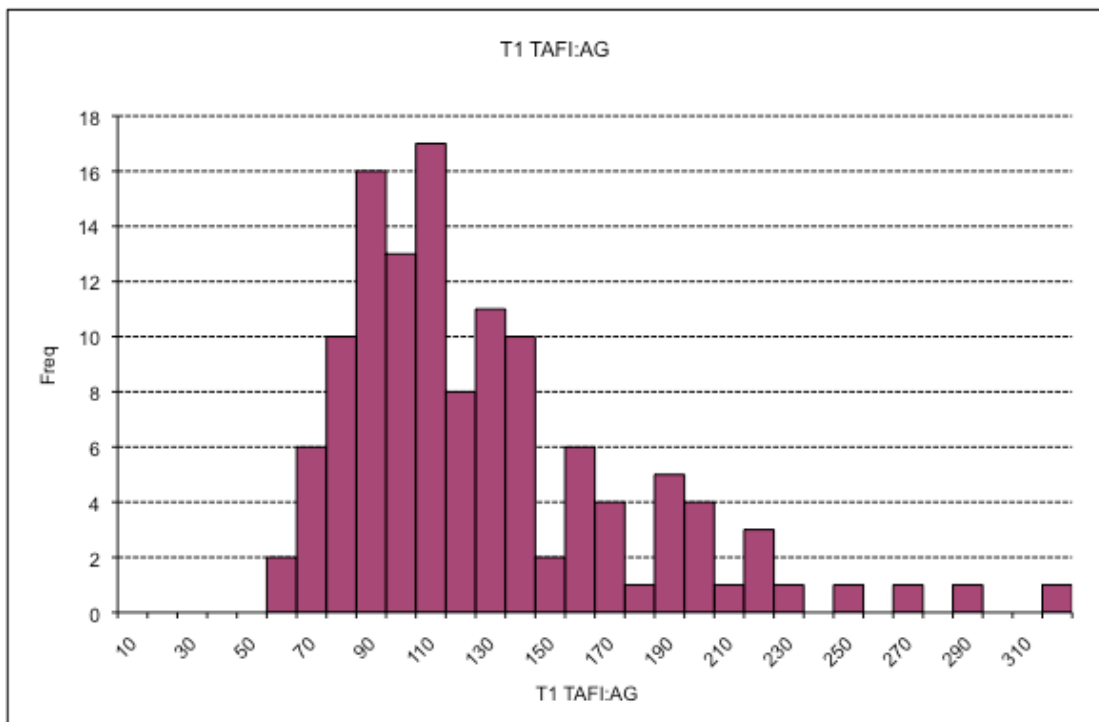


Figure 16: The distribution of TAFI Ag in the second trimester

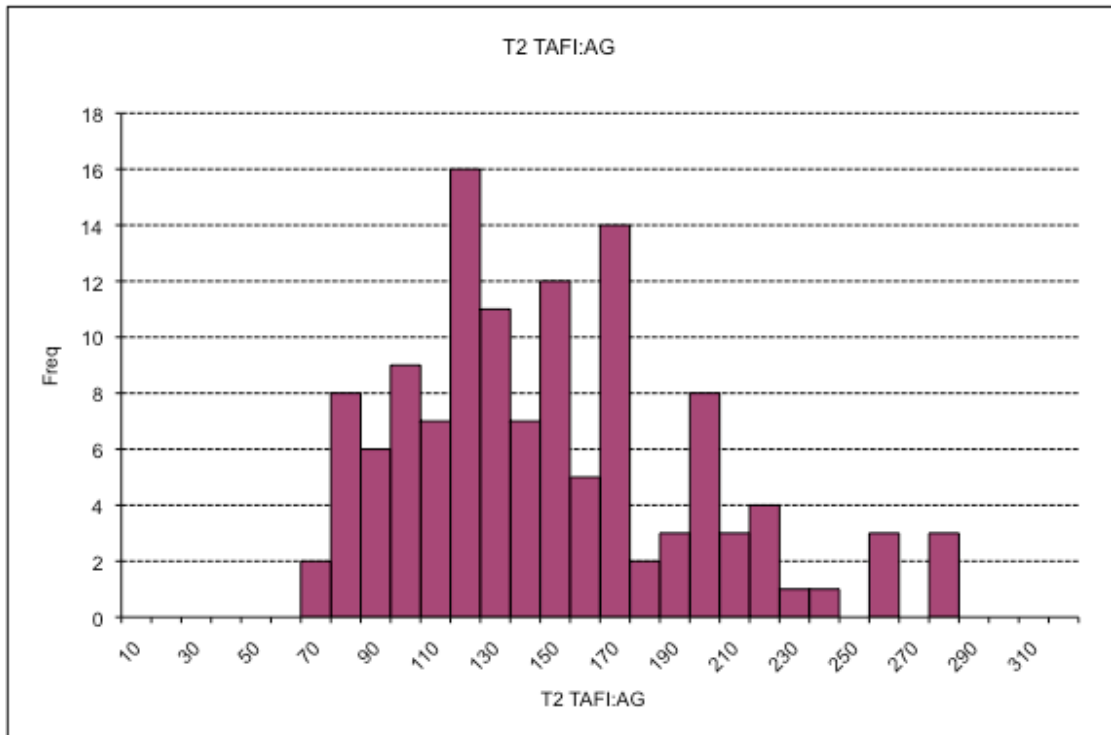
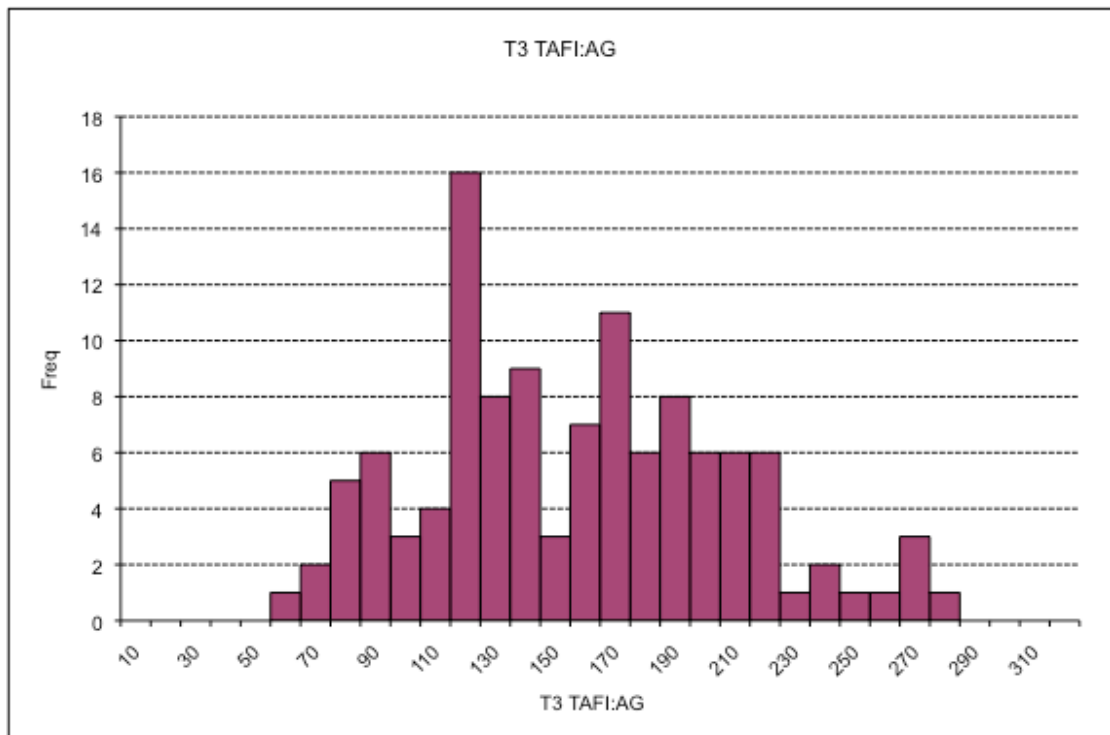


Figure 17: The distribution of TAFI Ag in the third trimester



4.3.4 CLT in the presence of Buffer and PTCI:

The contribution of TAFI to the impairment of fibrinolysis was quantified by assessing the difference in CLT after the addition of a specific TAFIa inhibitor (PTCI). CLT in the presence of PTCI was significantly shorter in all trimesters, confirming that TAFI's contribution to the inhibition of fibrinolysis.

Table 10: Contribution of TAFIa to fibrinolysis as assessed by the difference between CLT in the presence of buffer and the presence of PTCI in normal pregnancy

	Median CLT in the presence of buffer (min)	Median CLT in the presence of PTCI (min)	P value for the difference between the medians*
Trimester 1	68	51	<0.001
Trimester 2	82	66	<0.001
Trimester 3	93	72	<0.001

*Significance was assumed <0.05 using Wilcoxon sum rank test

4.3.5 Correlation between TAFI and CLT

The correlation between CLT and TAFI Ag and TAFIa levels was also investigated. There was a general trend for longer CLT with higher levels of both TAFI Ag and TAFIa. No significant positive correlation between CLT and TAFI Ag was seen at any gestation.

Table 11: Correlation between TAFIag and TAFIa with CLT in each trimester

	Pearson's correlation r ²	P value
TAFIag and CLT in T1	0.170	0.229
TAFIag and CLT in T2	0.030	0.833
TAFIag and CLT in T3	-0.213	0.126
TAFIa and CLT in T1	-0.050	0.730
TAFIa and CLT in T2	-0.059	0.686
TAFIa and CLT in T3	-0.213	0.756

Figure 18a: Correlation between TAFI Ag and CLT in the first trimester

($r=0.170$)

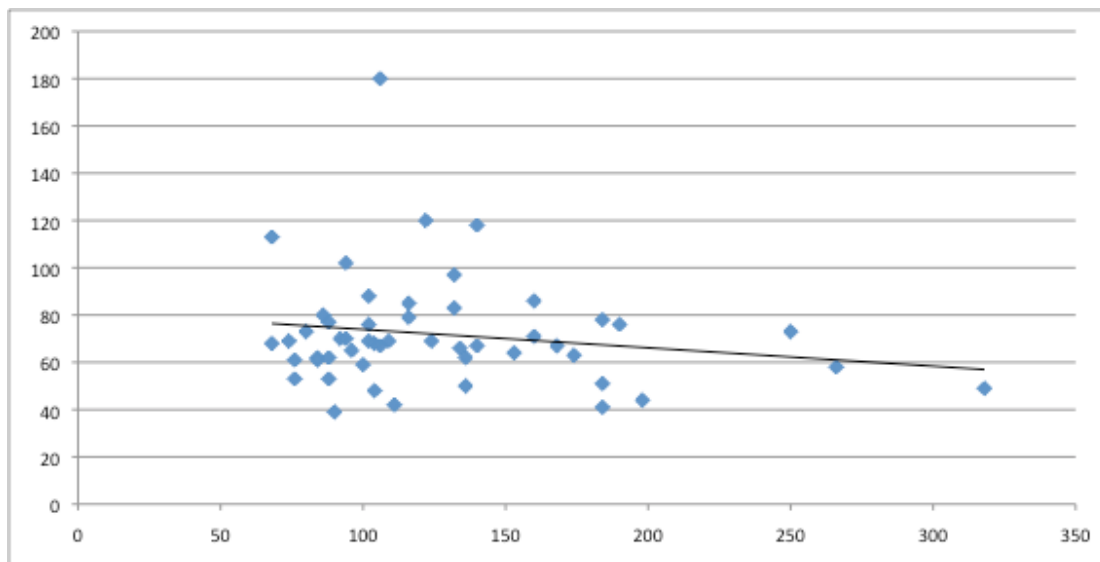


Figure 18b : Correlation between TAFIa and CLT in the first trimester

($r=0.050$)

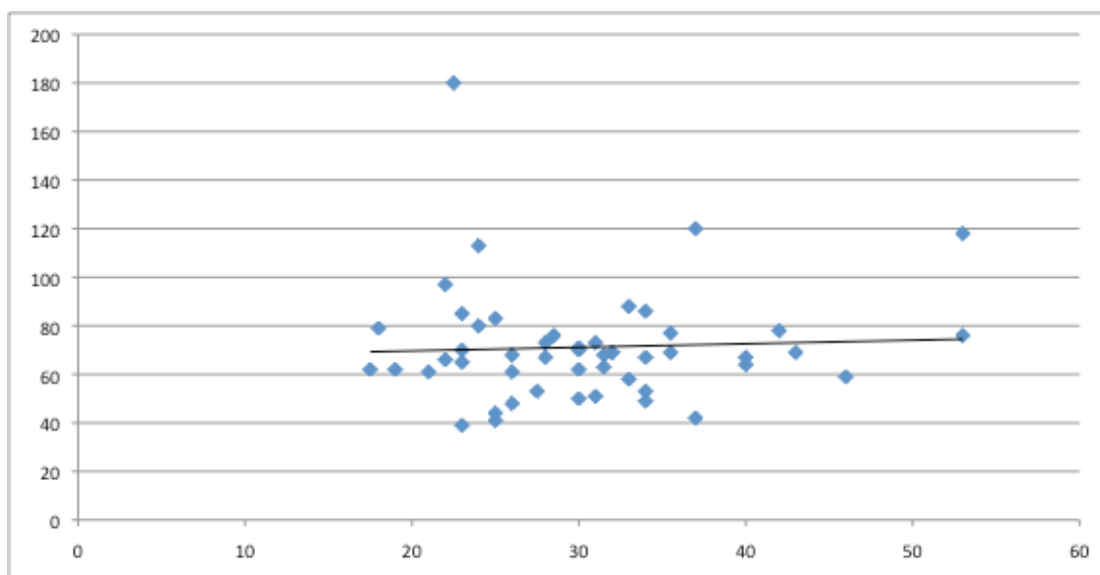


Figure 19a: Correlation between TAFI Ag and CLT in the second trimester

($r=0.030$)

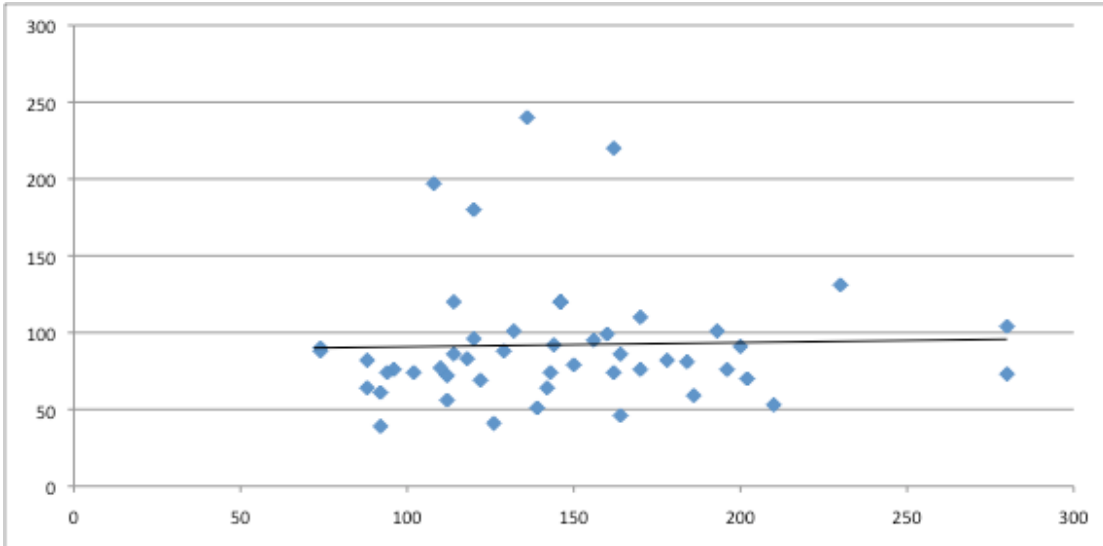


Figure 19b : Correlation between TAFIa and CLT in the second trimester

($r= -0.059$)

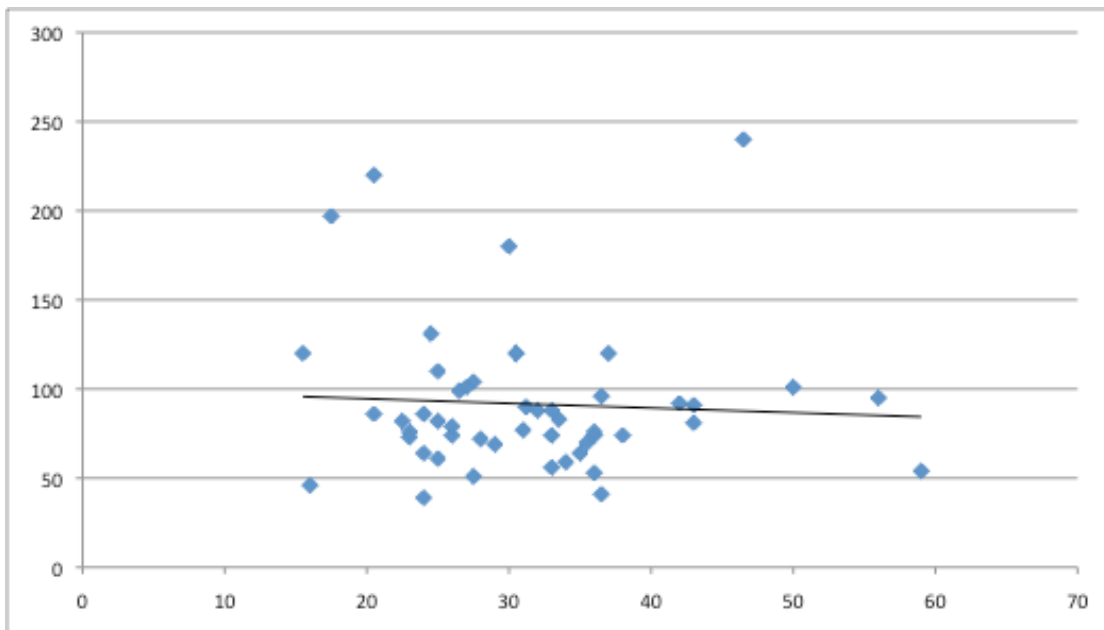


Figure 20a : Correlation between TAFIa_g and CLT in the third trimester (r=-0.213)

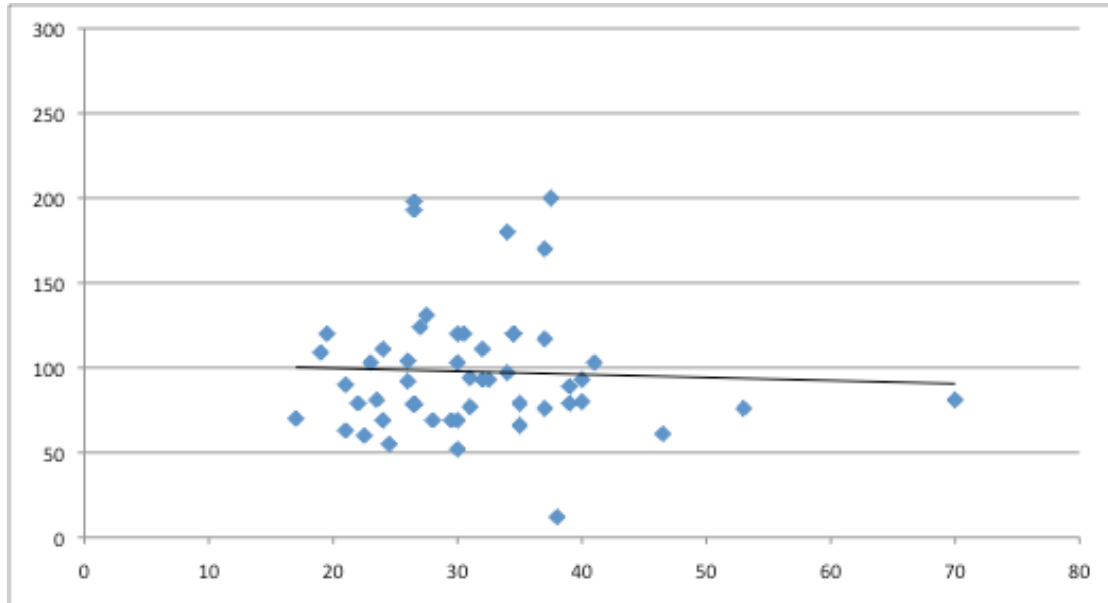
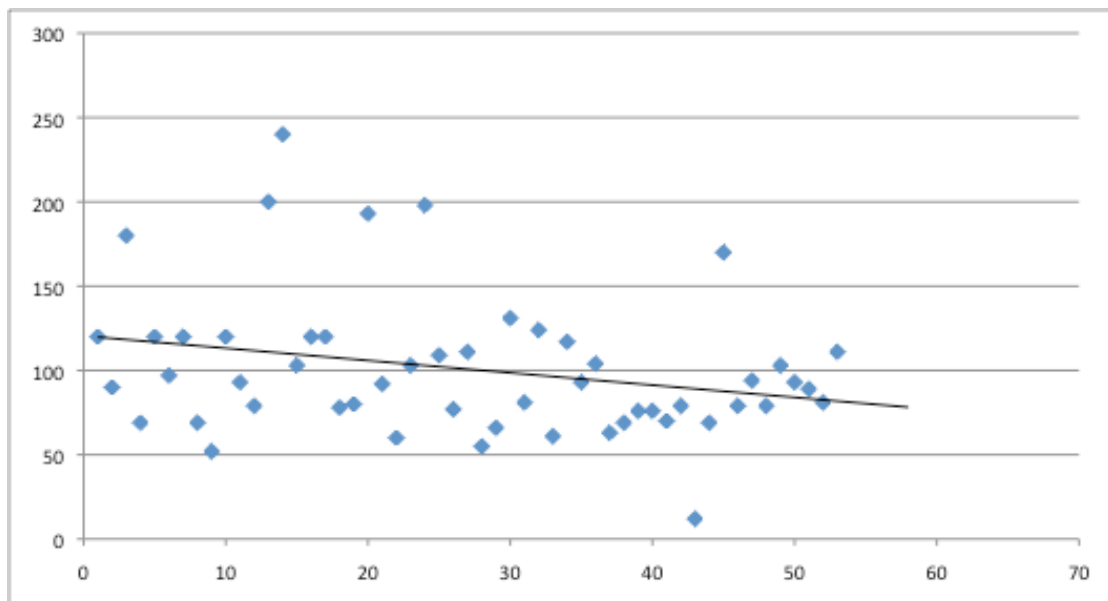


Figure 20b: Correlation between TAFIa_a and CLT in the third trimester (r=-0.044)



4.4 Discussion:

The present study is unique amongst published data, in that it investigates the nature of changes in TAFI during pregnancy using TAFI Ag, TAFIa and CLT in a longitudinal manner. Furthermore, it is the largest prospective longitudinal study to date including 127 uncomplicated pregnancies and the first to provide a reference range for TAFI Ag and TAFIa. We have demonstrated that TAFI levels increase with gestation with levels, plateauing in the third trimester. There are significant increases in TAFI Ag, TAFIa and CLT between the first and second trimesters and the first and third trimesters. A general trend for longer CLT was seen with higher levels of TAFI Ag and TAFIa. In addition, the contribution of TAFI to the impairment of fibrinolysis was confirmed by significantly shorter CLT seen at every gestation when the assay was performed in the presence of PTCI

There are contrasting reports of TAFI levels during pregnancy. Three out of the four published studies considering TAFI Ag and TAFIa in normal pregnancy demonstrate a significant progressive increase from early pregnancy (Chabloz, Reber et al. 2001; Watanabe, Wada et al. 2001; Mousa, Downey et al. 2004). Chabloz et al performed a cross sectional cohort study assessing TAFI Ag and D-dimer levels in 144 women with uncomplicated singleton pregnancies (Chabloz, Reber et al. 2001) and reported a steady significant increase in TAFI Ag levels between 10 and 25 weeks gestation, after which, TAFI Ag levels plateaued until they started to fall after delivery (Chabloz, Reber et al. 2001). These results support the findings of the current study that demonstrated a significant increase in TAFI Ag and TAFIa between the first and second trimesters.

A more recent cross-sectional case control study by Watanabe et al focused on changes in TAFIa during pregnancy (Watanabe, Wada et al. 2001). This prospective study demonstrated significantly higher TAFIa in 176 women with normal pregnancies compared to 15 non-pregnant women and confirmed a progressive increase in TAFIa starting in the first trimester, continuing up to 20 weeks after which levels remained steady until they started to decrease postnatally (Watanabe, Wada et al. 2001). This is one of the few studies that examines TAFI activity in normal pregnancy (Watanabe, Wada et al. 2001). However, it was cross-sectional in design, whereas in the current study is longitudinal, examining the changes in TAFI Ag and TAFIa in 130 women in each trimester of their pregnancies.

Another cross sectional study of 183 normal pregnancies measured TAFI Ag levels at four weekly intervals and reported a progressive increase in TAFI Ag throughout pregnancy, maximal at 35-39 weeks gestation, and a gradual decline in the levels post delivery (Mousa, Downey et al. 2004). Although it did not report changes in TAFIa, this study did examine TAFI's functional effect by measuring CLT, both with and without the addition of a TAFI inhibitor. The changes in CLT mirrored those of TAFI Ag, increasing with gestation. Thus, there is a progressive impairment of fibrinolysis as pregnancy progresses. Addition of the TAFI inhibitor during the CLT assay abrogated the increase in CLT, suggesting that the decreased rate of fibrinolysis is attributable to TAFI (Mousa, Downey et al. 2004). Once more, these results support the findings of the current study that show that CLT progressively increases during pregnancy and that there is a degree of correlation between CLT and TAFI Ag.

Many previous studies have used assays that were inadequately validated, making interpretation of their results difficult. Thus, accurate comparison of data generated by different studies will not be possible until an international standard for TAFI Ag is defined. In this study, to overcome the difficulties resulting from each of the various assays, all three were performed on each sample giving a much clearer picture of changes in TAFI during pregnancy.

Although samples were collected from two different hospital sites, efforts were made to minimise heterogeneity between the methods by which the samples were prepared for analysis. The samples collected at Colchester General Hospital were spun on site and separated into individual aliquots before they were transported to the Royal Free Hospital for analysis. This ensured that all samples were prepared in the same manner before their analysis at the Royal Free Hospital.

This study does have certain limitations. As this study does not include non-pregnant levels from the participants, it cannot confirm that TAFI Ag, TAFIa and CLT are raised in the first trimester or describe changes in TAFI post-partum

In conclusion, the data from this study provides reference ranges to TAFI Ag and TAFIa levels for the three trimesters of pregnancy. The study findings also supports previous reports of TAFI Ag, TAFIa and CLT increasing with gestation in normal pregnancy, with a significant increase in TAFI Ag and TAFIa levels between the first and second and first and third trimesters. Levels progressively increased throughout pregnancy. There was no significant change in TAFI Ag or TAFIa levels between the second and third trimesters, confirming the results of previous cross sectional studies.

It confirms the contribution of TAFI to the impairment of fibrinolysis as CLT in the presence of a specific TAFI inhibitor (PTCI) were significantly shorter at all gestations. This information can be used when examining the role of TAFI in pregnancy related complications, to elaborate on the pathogenesis of conditions such as PET.

Chapter 5

THROMBIN ACTIVATABLE FIBRINOLYSIS

INHIBITOR IN PRE ECLAMPSIA

5.1 Introduction:

As discussed in chapter 2, PET is characterised by impaired placental circulation and has been associated with impaired fibrinolysis (Perry and Martin 1992; Gilabert, Estelles et al. 1995; Schjetlein, Haugen et al. 1997; Sheppard and Bonnar 1999). Thus, TAFI may be involved in the abnormal haemostasis seen in women with PET.

This study aims to compare TAFI levels in women with PET to the reference ranges created in the previous chapter to evaluate TAFI's contribution to the pathophysiology of PET.

5.2 METHODS AND MATERIALS:

5.2.1 Study design:

This was a prospective cohort study of women who attended the Obstetric units of two hospitals (Royal Free Hospital and Colchester General Hospital). Two sample populations were included. The control group were the previously described in Chapter 4: a cohort of 127 women with uncomplicated singleton pregnancies from whom samples were collected in each trimester of their pregnancy. The study protocol was reviewed and approved by the Royal Free Hospital Ethics committee (see appendix 1). The case cohort consisted of 46 women diagnosed with PET (18 women from Colchester General hospital and 28 from the Royal Free Hospital). These women were recruited from antenatal clinic, the maternity Day Assessment Unit or labour ward after a diagnosis of PET had been confirmed. Thirty-six women were diagnosed with PET in the third trimester, and nine in the second trimester. Of the nine women diagnosed in the second trimester, only three remained pregnant after 28 weeks gestation. Samples were collected in both the second and third trimesters of women who

developed PET before 28 weeks and remained pregnant in the third trimester. Written informed consent was obtained from all patients and controls.

5.2.2 Inclusion and Exclusion criteria:

PET was defined as gestational hypertension of at least 140/90 mmHg on two separate occasions at least ≥ 4 hours apart associated with significant proteinuria of at least 300mg in a 24-hour urine collection. Only women with a singleton pregnancy were approached. Women with a pre-pregnancy history of: thrombophilia; thromboembolic disease; systemic lupus erythematosus; recurrent miscarriage; antiphospholipid syndrome; diabetes mellitus; hypertension; acute or chronic renal failure or other significant medical co-morbidity were excluded from the study. None of the participants were receiving non-steroidal anti-inflammatory, anti-platelet or anticoagulant therapy at the time of sample collection.

5.2.3 Data collection:

The following demographic data was collected prospectively for all study subjects: maternal age; parity; blood pressure and weight at antenatal booking visit and smoking history.

Pregnancy gestation was calculated using the dating scan performed between 11-13+6 weeks.

5.2.4 Blood collection

Sample collection was performed in the sitting position by direct venepuncture and collected into citrated tubes. Plasma samples were then prepared from citrated whole blood centrifuged at 2500g for 20 minutes, divided into aliquots and frozen at -70°C

until analysis. Samples obtained at Colchester General Hospital were centrifuged and divided into aliquots prior to being transported to the Royal Free Hospital Haemophilia Centre laboratory for analysis. TAFI antigen (TAFI Ag), TAFI activity (TAFIa) and clot lysis time (CLT) assays were then performed.

5.2.5 Laboratory Method

Each collected sample was used to determine TAFI Ag, TAFIa and CLT with and without the addition of PTCl. Analysis of the samples was performed as previously described in chapter 4. TAFI Actichrome®, TAFI Antigen Concentration (ELISA) and Clot lysis time were employed to determine TAFIa, TAFI Ag and CLT respectively.

5.2.6 Statistics

Descriptive statistics were utilised for demographic data. Continuous data were presented as mean and standard deviation. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software (SPSS Inc. Released 2009. PASW Statistics for windows, Version 18.0. Chicago:SPSS Inc).

In order to determine if the collected data had a normal distribution both visual and statistical methods were employed. A histogram created using SPSS demonstrated that the data was not normally distributed. This was then confirmed using the Shapiro-Wilk test in SPSS. Both methods demonstrated that the data did not have a normal distribution, hence non-parametric tests were used in the analysis. Comparisons between trimesters were performed using the Wilcoxon rank test. Spearman correlation was used for correlation analysis. A p value of <0.05 was accepted as statistically significant.

5.3 RESULTS:

Forty-three women were recruited to participate in the PET arm of the study. A further three women who were originally recruited to as part of the control group developed PET in the third trimester. Their results were included in the PET group. The numbers of samples collected in each trimester for the PET group are described in the table below.

Table 12: Number of women with PET recruited in each trimester

Trimesters in which samples collected	Number of women from whom samples collected (total n=48)
Trimesters 1, 2 and 3	3
Trimesters 2 and 3	3
Trimester 2 only (delivery prior to 28 weeks)	6
Trimester 3 (PET diagnosed after 28 weeks)	36

5.3.1 Demographic data:

The demographic data for the study participants is presented in table 12. The median age and parity of the participants were 28 (range=18-40) years and 0 (range 0-4) respectively.

Table 13: Distribution of age, parity and booking weight and blood pressure in women with PET

	Participants (n=46)
Parity:	
Unknown	0 (0 %)
0	36 (78 %)
1	6 (13%)
2	3 (7%)
3+	1 (2%)
Smoker	
Unknown	0 (0 %)
Yes	2 (4 %)
No	44 (96 %)
Age (years):	
Median (range)	28 (18-40)
Booking weight (kg)	
Median (range)	74.5 (46-110)
Systolic Blood Pressure at booking (mmHg)	
Median (range)	118 (90-138)
Diastolic Blood Pressure at Booking (mmHg)	
Median (range)	76 (60-98)

5.3.2 Changes in mean levels in the PET group:

TAFI Ag:

Mean TAFI Ag levels increased between the first and second trimesters and then dropped between the second and third. This was mirrored by the changes in Median TAFI Ag levels in women with PET- levels increased between the first and second trimesters and fell between the second and third trimesters.

Mean TAFI Ag levels were lower in the third trimester than the second trimester.

However, this change was not statistically significant ($p=0.25$).

TAFIa:

Mean TAFIa increased with gestation with the highest levels seen in the third trimester.

Median TAFIa levels also increased with gestation with the highest levels seen in the third trimester.

The change in mean TAFIa with between the second and third trimester was not statistically significant (p=0.24).

CLT:

Both mean and median CLT increased with gestation with the longest times seen in the third trimester.

The increase in mean CLT between the second and third trimesters achieved statistical significance (p=0.039).

Table 14a: Changes in TAFI Ag, TAFIa and CLT in women with PET

	Mean	Median (range)
TAFI Ag (%) Trimester 1	132.3	131 (100-161)
TAFI Ag (%) Trimester 2	198.0	204 (96-244)
TAFI Ag (%) Trimester 3	176.2	164 (74-280)
TAFIa (mcg/ml) Trimester 1	19.5	19.5 (18-21)
TAFIa (mcg/ml) Trimester 2	24.4	24 (14-38)
TAFIa (mcg/ml) Trimester 3	28.2	26 (7-61)
CLT (min) Trimester 1	67	67 (66-68)
CLT (min) Trimester 2	74.6	82 (45-97)
CLT (min) Trimester 3	118.2	120 (65-240)

Table 14b: Statistical significance of changes in the mean values of TAFI antigen, activity and clot lysis time between trimesters

	Significance of difference between means in Trimesters 2 and 3
TAFI Antigen in PET group	0.25
TAFI activity in PET group	0.24
CLT in PET group	0.039*

**denotes statistical significance where $p < 0.05$*

Figure 21: The distribution of TAFI Ag in the second and third trimesters in PET

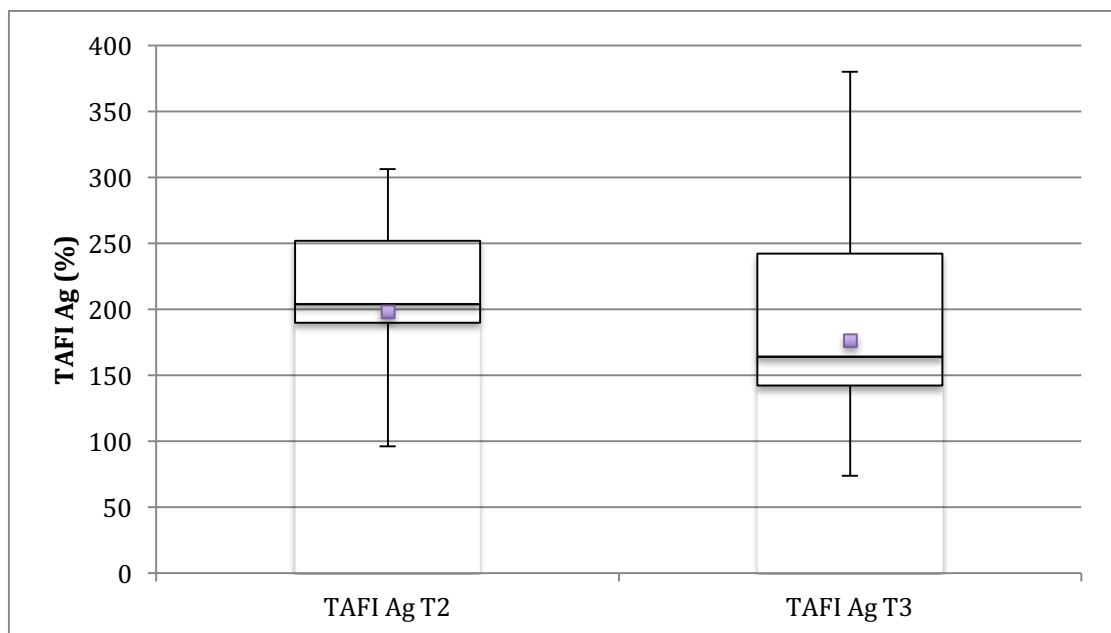


Figure 22: The distribution of TAFIa in the second and third trimesters in PET

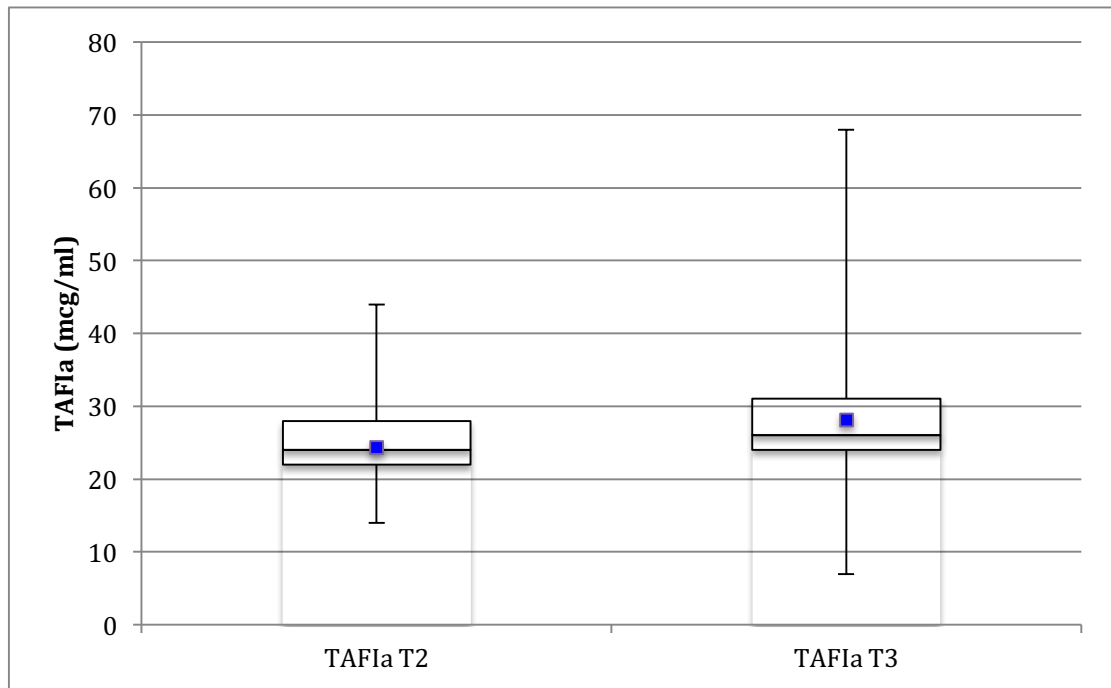
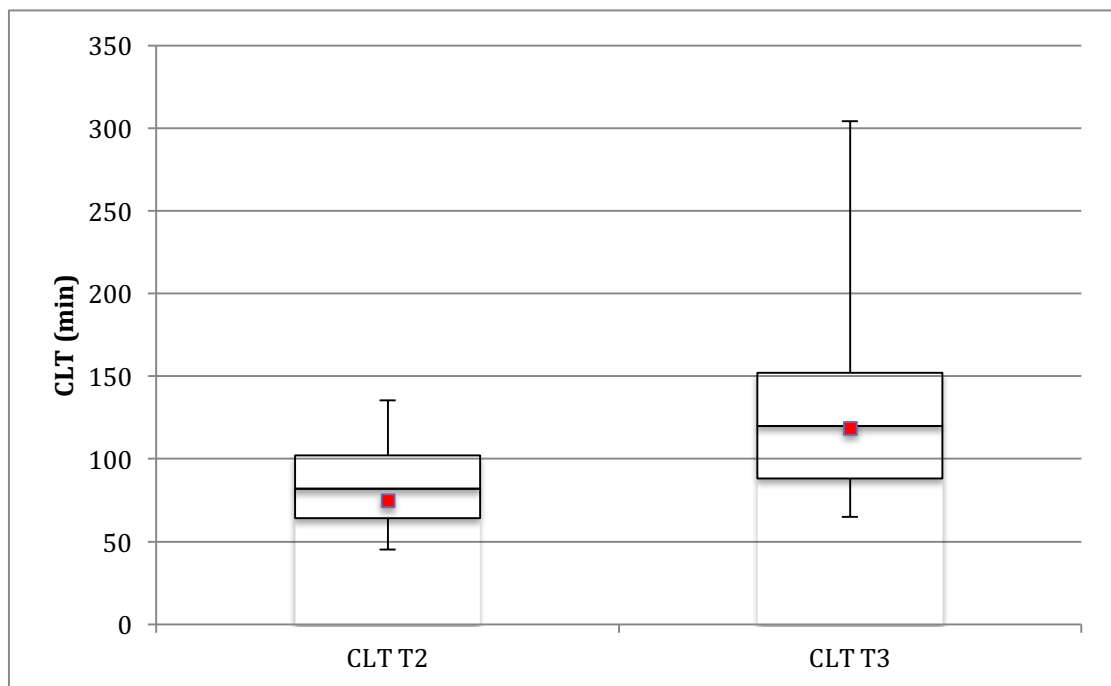


Figure 23: The distribution of CLT in the second and third trimesters in PET



5.3.3 Comparison of PET and normal pregnant controls:

TAFI Ag:

Mean TAFI Ag levels were significantly higher in the PET group when compared with normal pregnancy in the second and third trimesters ($p=0.002$ and $p=0.012$ respectively).

TAFIa:

Mean TAFIa levels were not found to be significantly different in the PET group when compared with the normal pregnant population ($p=0.25$ and $p=0.29$ in the second and third trimesters respectively).

CLT:

Mean CLT in the third trimester was not significantly higher in the PET group than in normal pregnancy ($p=0.055$). Additionally, there was no significant difference in CLT between the PET group and normal pregnant population seen in the second trimester ($p=0.376$).

Table 15: Comparison of Mean TAFI Ag, TAFIa and CLT in PET group and normal pregnant controls

	Mean in PET	Mean in Normal Pregnancy	P value for the difference between means*
TAFIag 2 nd Trimester	198.0	143.5	0.002*
TAFIag 3 rd Trimester	176.2	152.7	0.012*
TAFIa 2 nd Trimester	24.4	28.0	0.265
TAFIa 3 rd Trimester	28.2	29.8	0.344
CLT 2 nd Trimester	74.6	87.02	0.376
CLT 3 rd Trimester	118.2	99.6	0.055

**Calculated using independent samples t-test*

Three women who were initially recruited into the control group developed PET. Their results are displayed in the graphs below.

There was a trend for higher mean levels of TAFI Ag; lower TAFIa levels and longer CLTs in this small sample of women when compared with the control group.

Figure 24a: Mean TAFI Ag in each trimester in women with PET

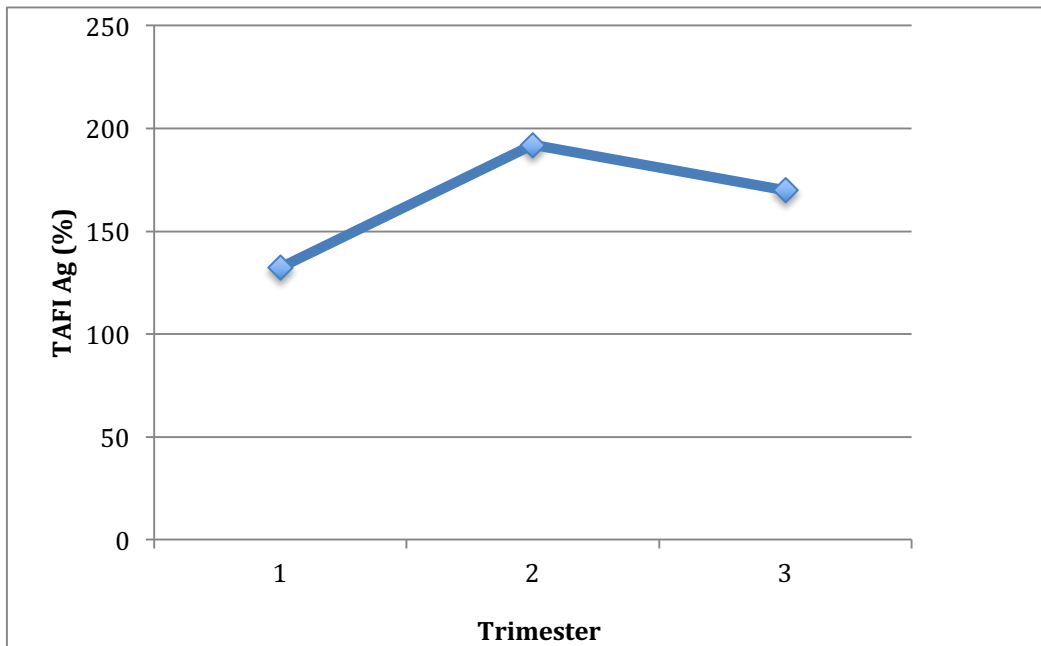


Figure 24b: Mean TAFIa in each trimester in women with PET

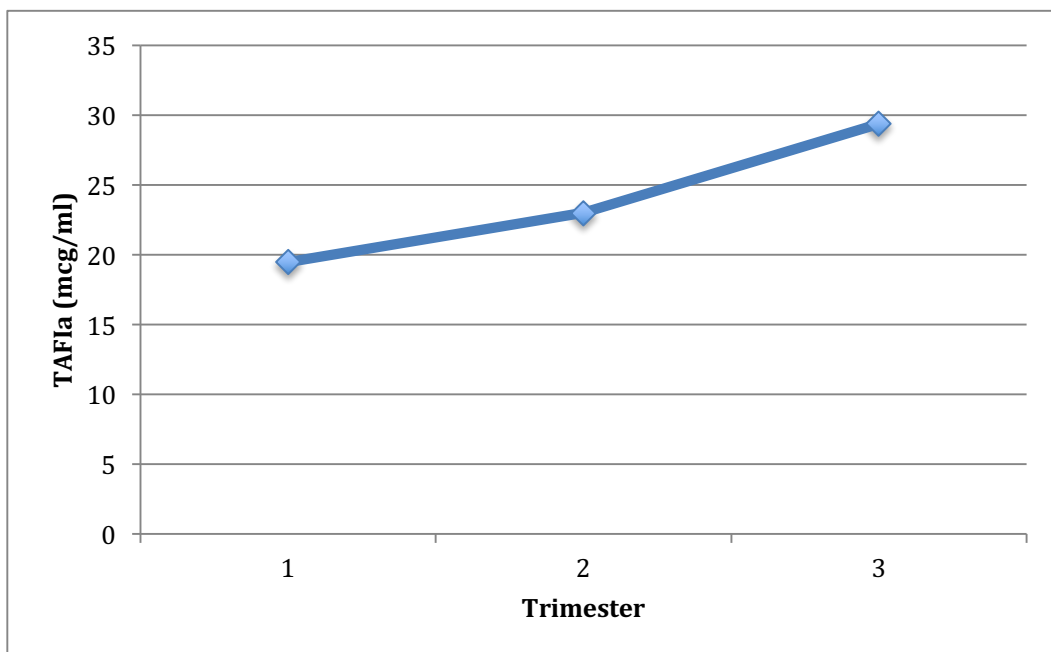
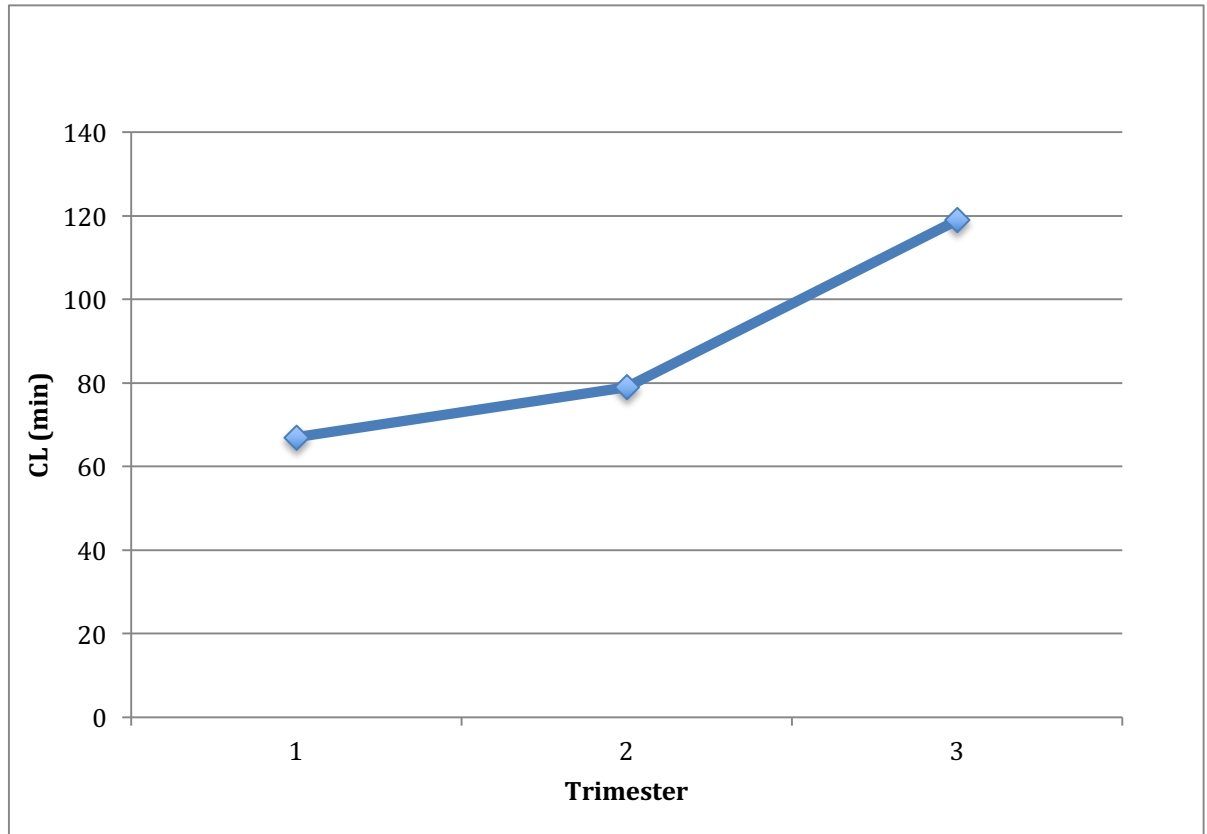


Figure 24c: Mean CLT in each trimester in women with PET



5.3.4 CLT in the presence of Buffer and PTCI:

The contribution of TAFI to the impairment of fibrinolysis was quantified by assessing the difference in CLT after the addition of a specific TAFIa inhibitor (PTCI). CLT in the presence of PTCI was significantly shorter in all samples in both the control and PET groups.

Table 16: Contribution of TAFIa to fibrinolysis as assessed by the difference between CLT in the presence of buffer and the presence of PTCI in normal pregnancy and PET

	Median CLT in the presence of buffer (min)	Median CLT in the presence of PTCI (min)	P value for the difference between the medians*
Trimester 2 PET group	82	67.5	<0.001
Trimester 3 PET group	120	94	<0.001

*Significance was assumed <0.05 using Wilcoxon sum rank test

5.3.5 Correlation between TAFI and CLT in PET

The correlation between CLT and TAFI Ag and TAFIa levels was also investigated. There was a general trend for longer CLT with higher levels of both TAFI Ag and TAFIa. No significant correlation between CLT and TAFI Ag or TAFIa was seen at any gestation.

Table 17: Correlation between TAFIag and TAFIa with CLT in each trimester

	Pearson's correlation r2	P value
TAFIag and CLT in T2	-0.967	0.833
TAFIag and CLT in T3	-0.137	0.126
TAFIa and CLT in T2	0.103	0.686
TAFIa and CLT in T3	0.137	0.756

Figure 25: Correlation between TAFI Ag and CLT in the third trimester in women with PET($r=0.137$)

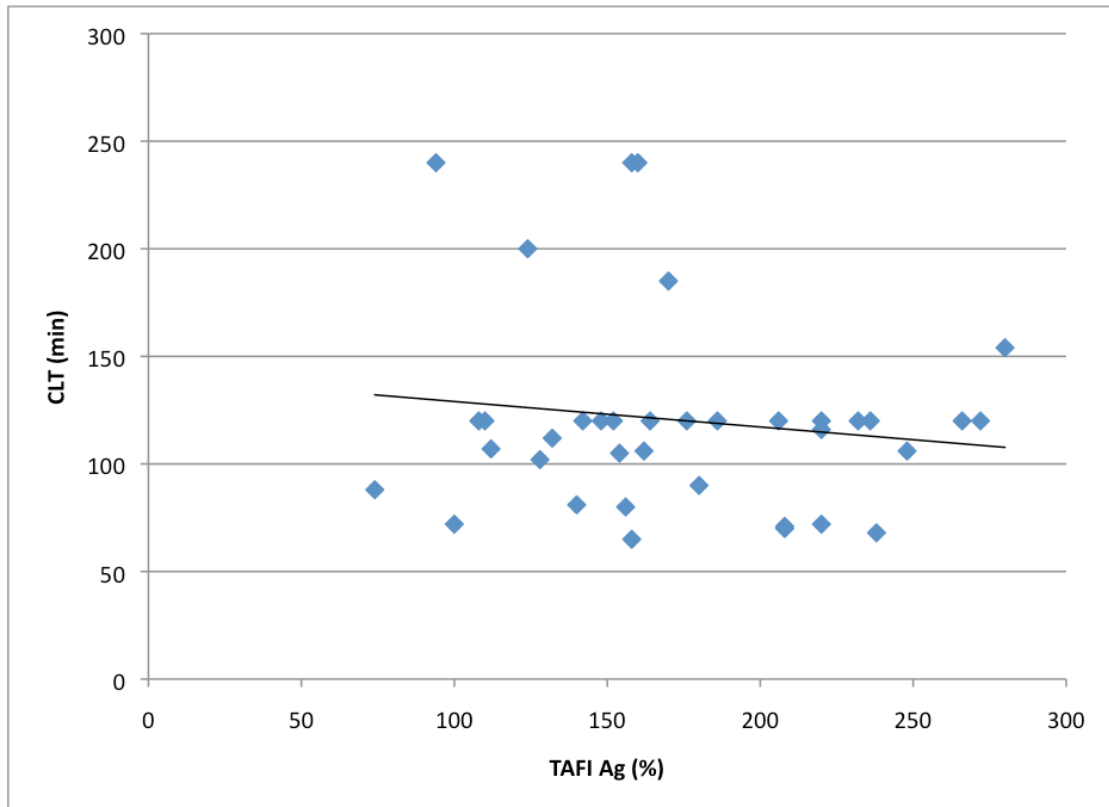
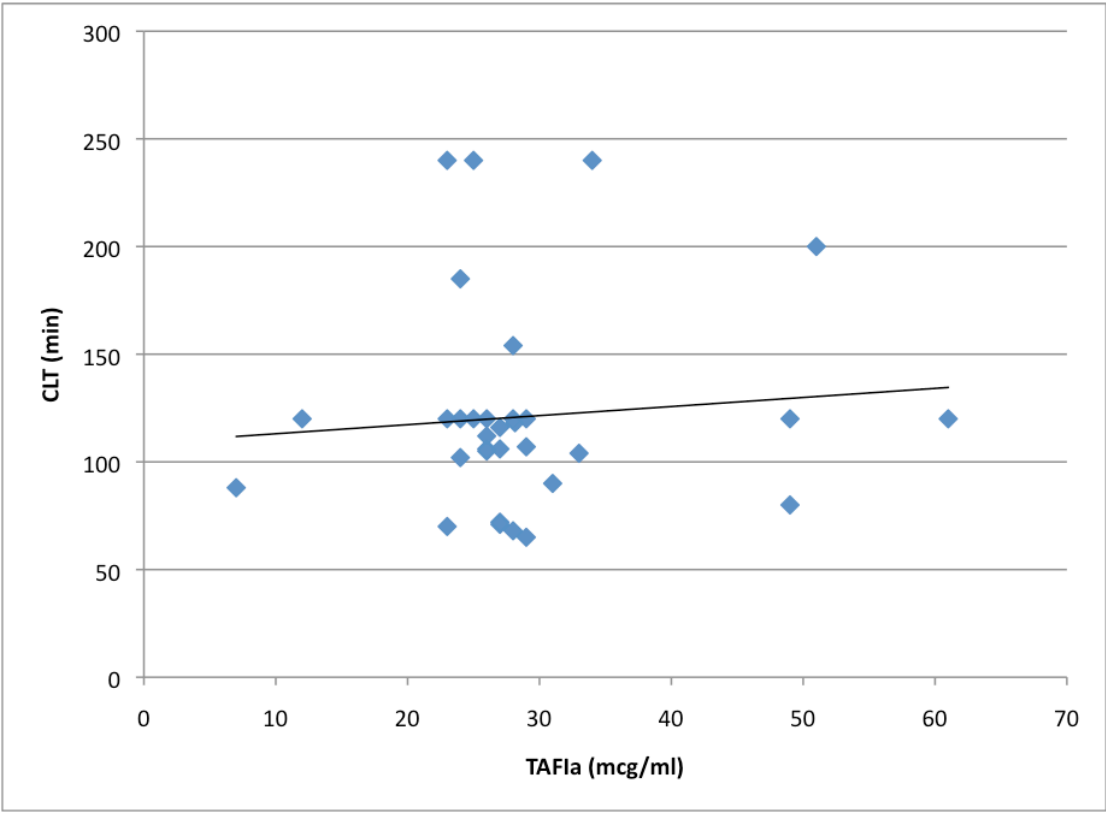


Figure 26: Correlation of TAFIa and CLT in the third trimester in women with PET (r=0.137)



5.4 DISCUSSION:

In the current study, significantly higher TAFI Ag levels were seen in the pre-eclamptic population. Additionally, TAFI Ag levels were higher in the second trimester than the third. This suggests that the impaired fibrinolysis seen in pre-eclamptic women may be attributed to higher TAFI Ag levels.

The clinical manifestations of PET can occur long after the abnormal placentation that precedes systemic endothelial dysfunction. This has led to the disease being described as a two stage disorder (Borzychowski AM 2006). Severe PET is more likely to present at an earlier gestation. We propose that the trend for higher TAFI Ag levels in the second trimester may contribute to further impairment of fibrinolysis, resulting in more severe disease, presenting earlier in pregnancy. We suggest that the lower TAFIa demonstrated in the PET group can be accounted for by the increased consumption of TAFI.

No significant difference was demonstrated between CLTs in the pre-eclamptic and general pregnant populations. This may be attributed to several factors: firstly, the small sample size of pre-eclamptic women in the second trimester (n=12). Secondly, the increased levels of TAFI Ag, lower levels of TAFa and shorter CLT seen in the second trimester in the PET group may be a result of more severe disease presenting at earlier gestations. TAFI Ag ELISA assays also quantify inactivated TAFIa. Thus, increased consumption of TAFIa leads to a corresponding shorter CLT and increased quantities of inactivated TAFIa fragments resulting in a higher measured TAFI Ag level.

The contribution of TAFI to the impairment of fibrinolysis in pre-eclamptic women is confirmed by the fact that CLTs in the presence of a TAFI inhibitor were significantly shorter than those performed with a buffer.

There are conflicting reports of TAFI Ag levels in pre-eclamptic women in the literature (Antovic, Rafik Hamad et al. 2002; Alacacioglu, Ozcan et al. 2004; Zhang, Hu et al. 2008; Martinez-Zamora, Tassies et al. 2010). Two of the four studies of TAFI Ag levels in women with PET compared to normal pregnant controls demonstrated higher levels in the PET group (Alacacioglu, Ozcan et al. 2004; Zhang, Hu et al. 2008). This supports the findings of the current study.

Antovic et al measured third trimester TAFI Ag levels in 46 women with PET (Antovic, Rafik Hamad et al. 2002) and reported significantly lower levels in PET compared to normal controls. The majority (63%) of their study population was composed of women with severe PET, which may account for the discordance with the results of the current study. The majority of our sample population had mild PET. Antovic et al attributed their findings to the impaired liver function and urinary excretion seen in women with severe PET.

This study does have limitations. Women were only recruited into the PET group after a diagnosis of PET had been confirmed. Thus, it was not possible to ascertain if higher levels of TAFI Ag seen in the PET group ante cede the manifestation of clinical symptoms. A large multicentre, longitudinal study would be able to collect this data. Furthermore, the PET group had a very small sample size of only three women. Thus, it does not provide enough data to compare early onset PET to late onset PET. It may be that severe, early onset PET has a different pathophysiology to PET at late

gestations. Finally, the current study assesses markers in the peripheral circulation and therefore does not evaluate haemostasis in the uteroplacental unit.

In conclusion, this is the only study to date that overcomes the various disadvantages of the different methodologies of quantifying TAFI by using multiple assays. The role of TAFI in the pathogenesis of PET could be confirmed by larger studies using the same methodology.

Chapter 6

OVERALL CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

6.1.1 Overall Conclusions:

This thesis includes the largest longitudinal study of TAFI in normal pregnancy. In addition it has provided pregnancy specific reference ranges for TAFI Ag and TAFIa. Both TAFI Ag and TAFIa levels were seen to increase with gestation with significant increases between levels in the first and second and first and third trimesters. TAFI's contribution to the impaired fibrinolysis characteristic of pregnancy was confirmed by significantly shorter CLTs after the addition of a specific TAFI inhibitor at all gestations. By quantifying both TAFI Ag and TAFIa levels and TAFI's contribution to altered haemostasis in pregnancy using CLT with and without PTCI, this study overcomes the difficulties introduced by the individual methods of measuring TAFI. By generating a pregnancy specific reference range for TAFI, this study provides the basis for larger studies examining the role of TAFI in complications of pregnancy, including recurrent miscarriage, hypertensive disorders and IUGR.

Significantly higher levels of TAFI Ag were demonstrated in pre-eclamptic women compared with the normal pregnant population. A trend for lower TAFIa levels in the PET group was also demonstrated. We propose that in addition to having a greater amount of TAFI Ag, a larger proportion of activated TAFI is consumed in the pre-eclamptic state leading to lower levels of fibrinolysis. This was supported by a trend for longer CLT seen in the PET group compared to normal controls. The findings of this study suggest that anti TAFI agents may have a role in the treatment or prevention of PET.

6.2 Suggestions for Future Studies

A larger multicentre prospective longitudinal study would be able to capture a large enough sample size to determine if raised TAFI Ag precedes the onset of clinical manifestations of PET. A sample size of approximately 1250 women would be required to capture data from 100 pre-eclamptic women. If TAFI Ag levels are raised in the first trimester it has the potential to be used as a screening test for PET, allowing for increased surveillance and early treatment in high-risk populations, leading to reduced maternal and fetal morbidity and mortality.

As described in previous chapters, the pathophysiology of PET has been studied extensively and as yet, remains undetermined. It is likely that PET has a multifactorial pathogenesis and that altered haemostasis is not the only mechanism at work. Therefore, patients at high risk of PET may be better identified using a combination of multiple predictors including uterine artery Doppler studies and other biomarkers for PET. This approach has been used successfully to screen for Down's syndrome and other trisomies in early pregnancy.

A woman's risk of developing PET can be classified using several different methods including: ultrasound; molecular markers in the maternal circulation; clinical history and booking blood pressure. Abnormal waveforms in the uterine artery Dopplers, demonstrated by a pulsatility index over the 95th centile and/ or notching predicts PET in high risk populations. Abnormal uterine artery Dopplers can identify 40% of women who go on to develop PET (Papageorghiou AT 2002). Maternal history at booking assessing for risk factors such as Afro-Caribbean race, a high body mass index, a personal or family history of PET (especially if early onset or severe disease) has been

shown to predict 47% of cases of PET and 35% of pregnancies affected by gestational hypertension (Poon LC 2010). The combination of maternal history and uterine artery Dopplers at 23 weeks gestation predict PET in 67.5% of women (Papergeorghiou AT 2005). However, due to a false positive rate of 25%, this model of prediction is of little value clinically (Papergeorghiou AT 2005). A large number of potential vascular biomarkers have been investigated as potential non-invasive methods of predicting PET. The most promising candidate molecular markers for pre-symptomatic PET include: inhibin A, PLGF, s-Flt 1, sEnd, VEGF and PAPP-A (Mone F 2014).

Akolekar et al recently described a model to predict PET using maternal characteristics, uterine artery Doppler pulsatility index, mean arterial pressure and biomarkers (serum pregnancy associated plasma protein and placental growth factor). Their study demonstrated a linear correlation between the components of their model and gestation age at delivery in women with PET that was able to predict 54% of all cases of PET (Akelokar R 2013). TAFI may prove to be part of a larger model to screen for PET in early pregnancy. A large statistical model of demographics, biomarkers including first trimester TAFI levels in conjunction with ultrasound parameters could form a robust screening method to predict the severity and onset of PET.

At present, all women in the UK are screened at booking for risk factors for PET, still birth and intrauterine growth restriction and PAPP-A is part of the combined screening test for chromosomal abnormalities. Women who are deemed to be at high risk of pregnancy complications due to placental insufficiency are then referred for uterine artery dopplers in the second trimester. Further studies could use the available screening tests that are part of routine antenatal care. Thus the combination of TAFIa and TAFI Ag levels in the second trimester with maternal history, uterine artery

dopplers and PAPP-A, may prove an effective screening test for the development of PET which has a higher specificity than the methods currently available.

However, even if the above model proved to be highly sensitive and specific, it may not prove cost effective. The cost of TAFI Ag and TAFIa assays used in this thesis were £7.00 and £12.00 per participant respectively. The laboratory time and technician costs would also have to be considered. Each TAFIa and TAFI Ag assay took approximately four to five hours to perform. In addition, each kit would require calibration prior to its use. Factoring in all of these issues, producing a TAFIa and TAFI Ag level for a single patient would cost approximately £90.

EF6265 is a synthetic highly specific inhibitor of TAFIa that does not affect plasmin, thrombin or any other serine proteases (Muto Y 2009). Toxicological studies have demonstrated EF6265 as having an acceptable safety profile to be used as an injectable agent (Muto Y 2009). EF6265 has been used to determine TAFIa's role in sepsis. Animal studies show that post treatment with EF6265 there are decreased markers of end organ damage in endotoxic septic shock (Muto Y 2009). High dose antithrombin treatment improves clinical outcomes in patients with sepsis complicated with disseminated intravascular coagulation (DIC) in the critical care setting (Warren BL 2001). Specific TAFIa inhibitors would increase the pro-fibrinolytic response due to DIC without decreasing the amount of fibrin that could act as a co-factor for tPA mediated plasminogen activation. Additionally, the thrombin induced release of tPA would be maintained. This data could be extrapolated to the pre-eclamptic population and may improve outcomes in women with severe PET who develop DIC. EF6265 could be used to study the effects of TAFIa inhibition in hypertensive disorders of pregnancy.

TAFI may also have a role in the pathophysiology of other complications of pregnancy including intrauterine growth retardation, recurrent miscarriage and implantation failure after embryo transfer. There are contradictory reports of TAFI levels in reproductive failure (Folkeringa, Korteweg et al. 2009; Knol, Veeger et al. 2009; Masini, Ticconi et al. 2009; Martinez-Zamora, Creus et al. 2010; Pruner, Djordjevic et al. 2010; Legnani, Bovara et al. 2012). A sufficiently powered cross sectional study could compare TAFI levels in women with a history of recurrent miscarriage or recurrent implantation failure with women who had successfully carried uncomplicated pregnancies to term. If women with recurrent pregnancy loss have altered TAFI levels, TAFI inhibitors may have a role in the prevention of miscarriage and improve the outcome of assisted reproductive techniques.

The findings presented in this thesis contribute to the understanding of the origins of PET and provide a basis for the further work to predict the onset and severity of the disease as well as other obstetric complications that are attributed to disordered haemostasis.

APPENDIX 1: STUDY ETHICAL APPROV



Joint Research Office

Office Location:
R&D Office
Admin Corridor Room G649
Medical School Building

Postal Address:
Royal Free London NHS Foundation Trust
Pond Street
London NW3 2QG

Email: hameedah.bogle-dawoud@nhs.net Tel No. 020 7472 6163 ext: 38900 Fax No 020 7431 6209
Web-sites: www.royalfree.nhs.uk; www.ucl.ac.uk/iro

18th May 2012

Dr Rezan A Abdul-Kadir
Royal Free London NHS Foundation Trust
University Department Of Obstetrics &
Gynaecology
Pond Street
London
NW3 2QG

Dear Dr Abdul-Kadir

RFH R&D Ref: **8271**(Please quote in all correspondence)
REC Ref: **11/H1109/5**
Title: **Study of Factor XI, Factor XII, Thrombin activateable fibrinolysis inhibitor (TAFI) and platelet aggregation in pregnancy: a comparison of normal women and those affected by bleeding disorders.**

Thank you for registering the above study with the UCL/UCLH/RF Joint Research Office (RFH Trust Site). I am pleased to inform you that your study now has local R&D approval to proceed and recruit participants at Royal Free Hampstead NHS Trust.

Please note that all documents received have been reviewed and this approval is granted on the basis of the key documents provided which are ethically approved by the Research Ethics Committee:

Document	Date
REC approval and approved documents	24/02/2012

This R&D approval is conditional upon you complying with all requirements of the Research Ethics Committee notice of favourable opinion and other any relevant regulatory bodies.

Please find attached the conditions of the R&D approval and a reminder of your responsibilities as a researcher and ensure that both yourself and the research team are familiar with and understand the roles and responsibilities both as a team and individually.

Please do not hesitate to contact a member of the team with regards to assistance and guidance for your research.

Yours sincerely


Dr Adele K. Fielding & Dr Emma C Morris
Joint Directors of Research and Development
Royal Free Hampstead NHS Trust

REFERENCES

- 430, A. C. O. N. (2009). "American College of Obstetricians and Gynecologists. Preimplantation genetic screening for aneuploidy." Obstet Gynecol **113**: 766-767.
- (CMACE), C. f. M. a. C. E. (2011). "Saving Mothers' Lives. Reviewing maternal deaths to make motherhood safer: 2006-2008." BJOG **118**: 1-203.
- (NICE), N. I. f. H. a. C. E. (2011). "Hypertension in pregnancy. NICE clinical guideline 107." London RCOG press.
- Abdel Gader, A. M., A. A. Al-Mishari, et al. (2006). "Total and free tissue factor pathway inhibitor in pregnancy hypertension." Int J Gynaecol Obstet **95**(3): 248-253.
- Acosta-Tejeda, M., H. Baptista-Gonzalez, et al. (2011). "Association between genotype and plasma levels of thrombin-activated fibrinolysis inhibitor (TAFI) in the development of preeclampsia." Thromb Res **128**(4): e39-42.
- Aharon, A., N. Lanir, et al. (2005). "Placental TFPI is decreased in gestational vascular complications and can be restored by maternal enoxaparin treatment." J Thromb Haemost **3**(10): 2355-2357.
- Ahmad, S. S., R. Rawala-Sheikh, et al. (1992). "Components and assembly of the factor X activating complex." Semin Thromb Hemost **18**(3): 311-323.
- Akelokar R, S. A., Poon L, Wright D, Nicholaides K (2013). "Competing risks model in early screening for pre eclampsia by biophysical and biochemical markers." Fet Diagn Ther **33**: 8-15.
- Alacacioglu, I., M. A. Ozcan, et al. (2004). "Plasma levels of thrombin activatable fibrinolysis inhibitor in normal and preeclamptic pregnant women." Thromb Res **114**(3): 155-159.
- Alberry M, B. V., Soothill P (2011). "An update on pre-eclampsia prediction research." TOG **13**: 79-85.
- Altman, D., G. Carroli, et al. (2002). "Do women with pre-eclampsia, and their babies, benefit from magnesium sulphate? The Magpie Trial: a randomised placebo-controlled trial." Lancet **359**(9321): 1877-1890.
- Antovic, J. P., R. Rafik Hamad, et al. (2002). "Does thrombin activatable fibrinolysis inhibitor (TAFI) contribute to impairment of fibrinolysis in patients with preeclampsia and/or intrauterine fetal growth retardation?" Thromb Haemost **88**(4): 644-647.
- Astedt, B., I. Hagerstrand, et al. (1986). "Cellular localisation in placenta of placental type plasminogen activator inhibitor." Thromb Haemost **56**(1): 63-65.
- Autiero, M., A. Luttmun, et al. (2003). "Placental growth factor and its receptor, vascular endothelial growth factor receptor-1: novel targets for stimulation of ischemic tissue revascularization and inhibition of angiogenic and inflammatory disorders." J Thromb Haemost **1**(7): 1356-1370.
- Aznar, J., J. Gilabert, et al. (1986). "Fibrinolytic activity and protein C in preeclampsia." Thromb Haemost **55**(3): 314-317.
- Bajzar, L., R. Manuel, et al. (1995). "Purification and characterization of TAFI, a thrombin-activable fibrinolysis inhibitor." J Biol Chem **270**(24): 14477-14484.

- Bajzar, L., J. Morser, et al. (1996). "TAFI, or plasma procarboxypeptidase B, couples the coagulation and fibrinolytic cascades through the thrombin-thrombomodulin complex." *J Biol Chem* **271**(28): 16603-16608.
- Barker, D. J., C. N. Martyn, et al. (1993). "Growth in utero and serum cholesterol concentrations in adult life." *BMJ* **307**(6918): 1524-1527.
- Barton, J. R. and B. M. Sibai (2008). "Prediction and prevention of recurrent preeclampsia." *Obstet Gynecol* **112**(2 Pt 1): 359-372.
- Bdolah, Y., C. Lam, et al. (2008). "Twin pregnancy and the risk of preeclampsia: bigger placenta or relative ischemia?" *Am J Obstet Gynecol* **198**(4): 428 e421-426.
- Beller, F. K. and C. Ebert (1982). "The coagulation and fibrinolytic enzyme system in pregnancy and in the puerperium." *Eur J Obstet Gynecol Reprod Biol* **13**(3): 177-197.
- Berks D, H. M., Raat H, Duvekot JJ, Steegers EA, Habbema JD. (2013). "Risk of cardiovascular disease after pre-eclampsia and the effect of lifestyle interventions: a literature based study." *BJOG* **120**: 924-931.
- Binette TM, T. F., Peer G, Bajzar L (2007). "Thrombin-thrombomodulin connects coagulation and fibrinolysis: more than an in vitro phenomenon." *Blood* **110**: 3168-3175.
- bjornsson, T., D. Schneider, et al. (1989). "Aspirin acetylates fibrinogen and enhances fibrinolysis. Fibrinolytic effect is independent of changes in plasminogen activator levels." *J Pharmacol Exp Ther* **250**: 154-161.
- Blom EA, J. P., Verhulst FC, et al (2010). "Perinatal complications increase the risk of postpartum depression. The Generation R Study." *BJOG* **117**: 1390-1398.
- Boffa, M. B., R. Bell, et al. (2000). "Roles of thermal instability and proteolytic cleavage in regulation of activated thrombin-activable fibrinolysis inhibitor." *J Biol Chem* **275**(17): 12868-12878.
- Boffa MB, K. M. (2007). "Curiouser and curiouser: recent advances in the measurement of thrombin activatable fibrinolysis inhibitor (TAFI) and in understanding its molecular genetics, gene regulation, and biological roles." *Clin Biochem* **40**: 431-442.
- Boffa, M. B., D. Maret, et al. (2008). "Effect of single nucleotide polymorphisms on expression of the gene encoding thrombin-activatable fibrinolysis inhibitor: a functional analysis." *Blood* **111**(1): 183-189.
- Boffa, M. C., L. Valsecchi, et al. (1998). "Predictive value of plasma thrombomodulin in preeclampsia and gestational hypertension." *Thromb Haemost* **79**(6): 1092-1095.
- Bonnar, J., G. P. McNicol, et al. (1971). "Coagulation and fibrinolytic systems in pre-eclampsia and eclampsia." *Br Med J* **2**(5752): 12-16.
- Borzychowski AM, S. I., Redman CW. (2006). "Inflammation and pre-eclampsia." *Semin Fetal Neonatal Med* **11**(5): 306-316.
- Bouma, B. N., P. F. Marx, et al. (2001). "Thrombin-activatable fibrinolysis inhibitor (TAFI, plasma procarboxypeptidase B, procarboxypeptidase R, procarboxypeptidase U)." *Thromb Res* **101**(5): 329-354.
- Bremme, K., E. Ostlund, et al. (1992). "Enhanced thrombin generation and fibrinolytic activity in normal pregnancy and the puerperium." *Obstet Gynecol* **80**(1): 132-137.
- Bremme, K. A. (2003). "Haemostatic changes in pregnancy." *Best Pract Res Clin Haematol* **16**(2): 153-168.

- Brenner, B. (2004). "Haemostatic changes in pregnancy." Thromb Res **114**(5-6): 409-414.
- Briceno-Perez C, B.-S. L., Vigil De Gracia P. (2009). "Prediction and prevention of pre-eclampsia." Hypertens Pregnancy **28**: 138-155.
- Broughton-Pipkin F, R. P. (1994). "Pre-eclampsia- the disease of theories." Br Med Bull **50**(2): 381-396.
- Brouwers, G., H. Vols, et al. (2001). "A novel, possibly functional, single nucleotide polymorphism in the coding region of the thrombin activatable fibrinolysis inhibitor (TAFI) gene is also associated with TAFI levels." Blood **98**: 1992-1993.
- Broze, G. J., Jr., L. A. Warren, et al. (1988). "The lipoprotein-associated coagulation inhibitor that inhibits the factor VII-tissue factor complex also inhibits factor Xa: insight into its possible mechanism of action." Blood **71**(2): 335-343.
- Buelens K, H.-G. G., Muyldermans S, Gils A, Declerck PJ (2010). "Generation and characterisation of inhibitory nanobodies towards thrombin activateable fibrinolysis inhibitor." J Thromb Haemost **8**: 1302-1312.
- Butenas, S., C. van 't Veer, et al. (1997). "Evaluation of the initiation phase of blood coagulation using ultrasensitive assays for serine proteases." J Biol Chem **272**(34): 21527-21533.
- Cadroy, Y., H. Grandjean, et al. (1993). "Evaluation of six markers of haemostatic system in normal pregnancy and pregnancy complicated by hypertension or pre-eclampsia." Br J Obstet Gynaecol **100**(5): 416-420.
- Campbell, W. and H. Okada (1989). "An arginine specific carboxypeptidase generated in blood during coagulation or inflammation which is unrelated to carboxypeptidase N or its subunits." Biochem Biophys Res Commun **162**(3): 933-939.
- Campbell, W. D., E. Lazoura, et al. (2002). "Inactivation of C3a and C5a octapeptides by carboxypeptidase R and carboxypeptidase N." Microbiol Immunol **46**(2): 131-134.
- Caniggia, I., C. V. Taylor, et al. (1997). "Endoglin regulates trophoblast differentiation along the invasive pathway in human placental villous explants." Endocrinology **138**(11): 4977-4988.
- Cantwell, R., T. Clutton-Brock, et al. (2011). "Saving Mothers' Lives: Reviewing maternal deaths to make motherhood safer: 2006-2008. The Eighth Report of the Confidential Enquiries into Maternal Deaths in the United Kingdom." BJOG **118 Suppl 1**: 1-203.
- Carmeliet, P., L. Moons, et al. (2001). "Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions." Nat Med **7**(5): 575-583.
- Carr, D. B., M. Epplein, et al. (2005). "A sister's risk: family history as a predictor of preeclampsia." Am J Obstet Gynecol **193**(3 Pt 2): 965-972.
- Caughey AB, S. N., Washington AE, Escobar GJ. (2005). "Maternal ethnicity, paternal ethnicity and parental ethnic discordance: predictors of pre-eclampsia." Obstet Gynecol **106**(1): 156-161.
- Ceresa, E., M. Peeters, et al. (2007). "Announcing a TAFIa mutant with a 180-fold increased half-life and concomitantly a strongly increased antifibrinolytic potential." J Thromb Haemost **5**(2): 418-420.
- Cernecka, F., G. Ricci, et al. (1997). "Coagulation and fibrinolysis changes in normal pregnancy. Increased levels of procoagulants and reduced levels of inhibitors

- during pregnancy induce a hypercoagulable state, combined with a reactive fibrinolysis." Eur J Obstet Gynecol Reprod Biol **73**(1): 31-36.
- Chabloz, P., G. Reber, et al. (2001). "TAFI antigen and D-dimer levels during normal pregnancy and at delivery." Br J Haematol **115**(1): 150-152.
- Chaiworapongsa, T., J. Yoshimatsu, et al. (2002). "Evidence of in vivo generation of thrombin in patients with small-for-gestational-age fetuses and pre-eclampsia." J Matern Fetal Neonatal Med **11**(6): 362-367.
- Chand, H. S., D. C. Foster, et al. (2005). "Structure, function and biology of tissue factor pathway inhibitor-2." Thromb Haemost **94**(6): 1122-1130.
- Chen, Y. (2009). "Novel angiogenic factors for predicting pre-eclampsia: sFlt-1, PlGF, and soluble endoglin." The open Clinical Chem J **2**: 1-6.
- Chetaille, P., M. C. Alessi, et al. (2000). "Plasma TAFI antigen variations in healthy subjects." Thromb Haemost **83**(6): 902-905.
- Christiansen, O., H. Nielsen, et al. (2006). "Future directions of failed implantation and recurrent miscarriage research." Reprod Biomed Online **13**: 71-83.
- Clark, D., C. Coulam, et al. (2001). "Unexplained recurrent miscarriage in the new millennium: a critical analysis of immune mechanisms and treatments." Hum Reprod Update **7**: 501-511.
- Clark, P. (2003). "Changes of hemostasis variables during pregnancy." Semin Vasc Med **3**(1): 13-24.
- Clark, P., J. Brenndand, et al. (1998). "Activated protein C sensitivity, protein C, protein S and coagulation in normal pregnancy." Thromb Haemost **79**(6): 1166-1170.
- Clark, P. and I. A. Greer (2003). "Prevention and treatment of arterial thrombosis in pregnancy." Semin Thromb Hemost **29**(2): 155-164.
- Clark, P., I. Walker, et al. (2010). "SPIN: the Scottish Pregnancy Intervention Study: a multicentre randomised controlled trial of low molecular weight heparin and low dose aspirin in women with recurrent miscarriage." Blood **115**: 4162-4167.
- Cnossen JS, V. K., de Vrieze N, ter Riet G, Mol BW, Franx A, et al. (2008). "Accuracy of mean arterial blood pressure and blood pressure measurements in predicting pre-eclampsia: a systematic review." BMJ **336**: 1117-1120.
- Colucci, M., A. Pentimone, et al. (2002). "Effect of heparin on TAFI dependent inhibition of fibrinolysis: relative importance of TAFIa generated by clot bound and fluid phase thrombin." Thromb Haemost **88**: 282-287.
- Colucci, M. and N. Semeraro (2012). "Thrombin activatable fibrinolysis inhibitor: at the nexus of fibrinolysis and inflammation." Thromb Res **129**: 314-319.
- Comeglio, P., S. Fedi, et al. (1996). "Blood clotting activation during normal pregnancy." Thromb Res **84**(3): 199-202.
- Comp, P. C., G. R. Thurnau, et al. (1986). "Functional and immunologic protein S levels are decreased during pregnancy." Blood **68**(4): 881-885.
- Coolman, M., C. J. de Groot, et al. (2006). "Concentrations of plasminogen activators and their inhibitors in blood preconceptionally, during and after pregnancy." Eur J Obstet Gynecol Reprod Biol **128**(1-2): 22-28.
- Coulam, C., R. Jeyendran, et al. (2006). "Multiple thrombophilic gene mutations are risk factors for implantation failure." Reprod Biomed Online **12**: 322-327.
- Cusimano MC, P. J., Roddy M, Cho CK, Smith GN (2014). "The maternal health clinic: an initiative for cardiovascular risk identification in women with pregnancy related complications." Am J Obstet Gynecol **210**(438): e1-9.

- Cvirn, G., S. Gallistl, et al. (2003). "Low tissue factor pathway inhibitor (TFPI) together with low antithrombin allows sufficient thrombin generation in neonates." J Thromb Haemost **1**(2): 263-268.
- Cvirn, G., S. Gallistl, et al. (1999). "Effects of antithrombin and protein C on thrombin generation in newborn and adult plasma." Thromb Res **93**(4): 183-190.
- de Boer, K., J. W. ten Cate, et al. (1989). "Enhanced thrombin generation in normal and hypertensive pregnancy." Am J Obstet Gynecol **160**(1): 95-100.
- De-Regil, P. C., Ansary A, Kulier R, Pena-Rosas JP. (2012). "Vitamin D supplementation for women during pregnancy." Cochrane Database Syst Rev(2): CD008873.
- Declercq, P. J. (2011). "Thrombin activatable fibrinolysis inhibitor." Hamostaseologie **31**(3): 165-166, 168-173.
- Dekker G, S. B. (2001). "Primary, secondary and tertiary prevention of pre-eclampsia." Lancet **357**: 209-215.
- Douglas, J. T., M. Shah, et al. (1982). "Plasma fibrinopeptide A and beta-thromboglobulin in pre-eclampsia and pregnancy hypertension." Thromb Haemost **47**(1): 54-55.
- Doyle LW, C. C., Middleton P, Marret S, Rouse D. (2009). "Magnesium sulphate for women at risk of preterm birth for neuroprotection for the fetus." Cochrane Database Syst Rev **1**: CD004661.
- Duckitt, K. and D. Harrington (2005). "Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies." BMJ **330**(7491): 565.
- Duley, L. (2009). "The global impact of pre-eclampsia and eclampsia." Semin Perinatol **33**(3): 130-137.
- Duley, L., D. J. Henderson-Smart, et al. (2007). "Antiplatelet agents for preventing pre-eclampsia and its complications." Cochrane Database Syst Rev(2): CD004659.
- Dusse, L. M., M. G. Carvalho, et al. (2006). "Tissue factor and tissue factor pathway inhibitor: a potential role in pregnancy and obstetric vascular complications?" Clin Chim Acta **372**(1-2): 43-46.
- Dusse, L. M., A. J. Cooper, et al. (2007). "Thrombin activatable fibrinolysis inhibitor (TAFI): a role in pre-eclampsia?" Clin Chim Acta **378**(1-2): 1-6.
- Easterling, T. R. (1992). "The maternal hemodynamics of preeclampsia." Clin Obstet Gynecol **35**(2): 375-386.
- Eaton, D. L., B. E. Malloy, et al. (1991). "Isolation, molecular cloning, and partial characterization of a novel carboxypeptidase B from human plasma." J Biol Chem **266**(32): 21833-21838.
- el-Lati SG, D. C., Church MK (1994). "Complement peptides C3a and C5a induced mediator release from dissociated human skin mast cells." J Invest Dermatol **102**: 803-806.
- England, L. J., R. J. Levine, et al. (2002). "Smoking before pregnancy and risk of gestational hypertension and preeclampsia." Am J Obstet Gynecol **186**(5): 1035-1040.
- Epstein, F. (1964). "Late vascular effects of Toxaemia of pregnancy." N Engl J Med **271**: 391-395.
- Eremina, V., J. A. Jefferson, et al. (2008). "VEGF inhibition and renal thrombotic microangiopathy." N Engl J Med **358**(11): 1129-1136.

- Eremina, V., M. Sood, et al. (2003). "Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases." J Clin Invest **111**(5): 707-716.
- Erez, O., F. Gotsch, et al. (2009). "Evidence of maternal platelet activation, excessive thrombin generation, and high amniotic fluid tissue factor immunoreactivity and functional activity in patients with fetal death." J Matern Fetal Neonatal Med **22**(8): 672-687.
- Erez, O., R. Romero, et al. (2008). "Tissue factor and its natural inhibitor in pre-eclampsia and SGA." J Matern Fetal Neonatal Med **21**(12): 855-869.
- Eriksson, J. G., T. Forsen, et al. (2001). "Early growth and coronary heart disease in later life: longitudinal study." BMJ **322**(7292): 949-953.
- Escobar, C. E., D. M. Harmening, et al. (2002). Introduction to hemostasis. Clinical Hematology and fundamentals of hemostasis. D. M. Harmening. Philadelphia, PA, PA:FA Davis Company: 441-470.
- Esmon, C. T. (1995). "Thrombomodulin as a model of molecular mechanisms that modulate protease specificity and function at the vessel surface." FASEB J **9**(10): 946-955.
- Esplin, M. S., M. B. Fausett, et al. (2001). "Paternal and maternal components of the predisposition to preeclampsia." N Engl J Med **344**(12): 867-872.
- Estelles, A., J. Gilabert, et al. (1998). "Abnormal expression of type 1 plasminogen activator inhibitor and tissue factor in severe preeclampsia." Thromb Haemost **79**(3): 500-508.
- Farina, A., A. Sekizawa, et al. (2008). "Gene expression in chorionic villous samples at 11 weeks' gestation from women destined to develop preeclampsia." Prenat Diagn **28**(10): 956-961.
- Faught, W., P. Garner, et al. (1995). "Changes in protein C and protein S levels in normal pregnancy." Am J Obstet Gynecol **172**(1 Pt 1): 147-150.
- Fay, R. A., A. O. Hughes, et al. (1983). "Platelets in pregnancy: hyperdestruction in pregnancy." Obstet Gynecol **61**(2): 238-240.
- Fleury, V. and E. Angles-Cano (1991). "Characterization of the binding of plasminogen to fibrin surfaces: the role of carboxy-terminal lysines." Biochemistry **30**(30): 7630-7638.
- Foley JH, K. P., Mutch NJ, Gils A (2013). "Insights into the thrombin activatable fibrinolysis inhibitor function and regulation." J Thromb Haemost **11**(Suppl. 1): 306-315.
- Foley JH, K. P., Nesheim ME (2008). "Thrombin activatable fibrinolysis inhibitor zymogen does not play a significant role in the attenuation of fibrinolysis." J Biol Chem **283**: 8863-8867.
- Folkeringa, N., F. Korteweg, et al. (2009). "Thrombin activatable fibrinolysis inhibitor (TAFI) is not associated with fetal loss, a retrospective study." Thromb Res **123**: 511-514.
- Franchini, M. (2006). "Haemostasis and pregnancy." Thromb Haemos **95**: 401-413.
- Frere, C., P. E. Morange, et al. (2005). "Quantification of thrombin activatable fibrinolysis inhibitor (TAFI) gene polymorphism effects on plasma levels of TAFI measured with assays insensitive to isoform-dependent artefact." Thromb Haemost **94**(2): 373-379.
- Gant, N. F., S. Chand, et al. (1974). "The nature of pressor responsiveness to angiotensin II in human pregnancy." Obstet Gynecol **43**(6): 854.
- Genbacev, O., E. DiFederico, et al. (1999). "Invasive cytotrophoblast apoptosis in pre-eclampsia." Hum Reprod **14 Suppl 2**: 59-66.

- Gilabert, J., A. Estelles, et al. (1995). "Fibrinolytic system and reproductive process with special reference to fibrinolytic failure in pre-eclampsia." Hum Reprod **10 Suppl 2**: 121-131.
- Gilabert, J., J. A. Fernandez, et al. (1988). "Physiological coagulation inhibitors (protein S, protein C and antithrombin III) in severe preeclamptic states and in users of oral contraceptives." Thromb Res **49**(3): 319-329.
- Gils, A., M. C. Alessi, et al. (2003). "Development of a genotype 325-specific proCPU/TAFI ELISA." Arterioscler Thromb Vasc Biol **23**(6): 1122-1127.
- Gils, A., E. Ceresa, et al. (2005). "Modulation of TAFI function through different pathways--implications for the development of TAFI inhibitors." J Thromb Haemost **3**(12): 2745-2753.
- Gonzalez, R., I. Alberca, et al. (1985). "Protein C levels in late pregnancy, postpartum and in women on oral contraceptives." Thromb Res **39**(5): 637-640.
- Granger, J. P., B. B. LaMarca, et al. (2006). "Reduced uterine perfusion pressure (RUPP) model for studying cardiovascular-renal dysfunction in response to placental ischemia." Methods Mol Med **122**: 383-392.
- Gregersen, P., L. L. Leung, et al. (2011). "Plasma carboxypeptidase B down regulates inflammatory responses in autoimmune arthritis." J Clin Invest **121**: 3517-3527.
- Gris, J., J. Schved, et al. (1990). "Impaired fibrinolytic capacity and early recurrent spontaneous abortion." BMJ **300**: 1500.
- Guimaraes, A. H., N. H. van Tilburg, et al. (2004). "Association between thrombin activatable fibrinolysis inhibitor genotype and levels in plasma: comparison of different assays." Br J Haematol **124**(5): 659-665.
- Guo, Y., I. Hernandez, et al. (2009). "Cavolin-1-dependent apoptosis induced by fibrin degradation products " Blood **113**: 4431-4439.
- Habli M, E. N., Wiebracht E, et al. (2009). "Longterm maternal and subsequent pregnancy outcomes 5 years after haemolysis, elevated liver enzymes and low platelets syndrome (HELLP)." Am J Obstet Gynecol **201**: 385e.
- Halligan, A., J. Bonnar, et al. (1994). "Haemostatic, fibrinolytic and endothelial variables in normal pregnancies and pre-eclampsia." Br J Obstet Gynaecol **101**(6): 488-492.
- Hansen AR, B. C., Folkman J, McElrath TF (2010). "Maternal preeclampsia predicts the development of bronchopulmonary dysplasia." J Pediatr **156**: 532-536.
- Hashimoto M, Y. T., Oiwa T, Watanabe S, Giddings JC, Yamamoto J (2002). "Enhancement of the endogenous plasminogen activator induced thrombolysis by argatroban and APC and its control by TAFI, measured in an arterial thrombolysis model in vivo using rat mesenteric arteries." Thromb Haemost **87**: 110-113.
- He, S., K. Bremme, et al. (1996). "Decrease in plasminogen activator inhibitor type 2 related more to placental function and intrauterine growth than to severity of preeclampsia." Hypertension in Pregnancy **15**(2): 171-182.
- Hellgren, M. and M. Blomback (1981). "Studies on blood coagulation and fibrinolysis in pregnancy, during delivery and in the puerperium. I. Normal condition." Gynecol Obstet Invest **12**(3): 141-154.
- Hendriks, D., S. Scharpe, et al. (1989). "Characterisation of a carboxypeptidase in human serum distinct from carboxypeptidase N." J Clin Chem Clin Biochem **27**(5): 277-285.

- Henry, M., H. Aubert, et al. (2001). "Identification of polymorphisms in the promoter and the 3' region of the TAFI gene: evidence that plasma TAFI antigen levels are strongly genetically controlled." Blood **97**(7): 2053-2058.
- Hermes W, F. A., van Pampus MG, et al. (2013). "Cardiovascular risk factors in women who had hypertensive disorders late in pregnancy: a cohort study." Am J Obstet Gynecol **208**: e1-8.
- Hertig, A., N. Berkane, et al. (2004). "Maternal serum sFlt1 concentration is an early and reliable predictive marker of preeclampsia." Clin Chem **50**(9): 1702-1703.
- Hillmayer K, V. R., De Mayer M (2008). "Discovery of novel mechanisms and molecular targets for the inhibition of thrombin activatable fibrinolysis inhibitor." J Thromb Haemost **6**: 1892-1899.
- Hladunewich M, K. S., Lafayette R. (2007). "Pathophysiology of the clinical manifestations of pre-eclampsia." Clin J Am Soc Neph **2**(3): 543-549.
- Holmes, V. A., J. M. Wallace, et al. (2002). "Tissue factor expression on monocyte subpopulations during normal pregnancy." Thromb Haemost **87**(6): 953-958.
- Holthe, M. R., A. C. Staff, et al. (2004). "Different levels of platelet activation in preeclamptic, normotensive pregnant, and nonpregnant women." Am J Obstet Gynecol **190**(4): 1128-1134.
- Hornyak, T. J. and J. A. Shafer (1992). "Interactions of factor XIII with fibrin as substrate and cofactor." Biochemistry **31**(2): 423-429.
- Hoylaerts, M., D. C. Rijken, et al. (1982). "Kinetics of the activation of plasminogen by human tissue plasminogen activator. Role of fibrin." J Biol Chem **257**(6): 2912-2919.
- Huang, S. J., C. P. Chen, et al. (2008). "Pre-eclampsia is associated with dendritic cell recruitment into the uterine decidua." J Pathol **214**(3): 328-336.
- Hunt, B. J., H. Missfelder-Lobos, et al. (2009). "Pregnancy outcome and fibrinolytic, endothelial and coagulation markers in women undergoing uterine artery Doppler screening at 23 weeks." J Thromb Haemost **7**(6): 955-961.
- Huq FY, K. R. (2011). "Management of pregnancy, labour and delivery in women with inherited bleeding disorders." Haemophilia **17**(Suppl 1): 20-30.
- Inbal, A. and L. Muszbek (2003). "Coagulation factor deficiencies and pregnancy loss." Thromb Haemost **29**: 171-174.
- Institute, C. a. L. S. (2008). "Defining, establishing and verifying reference intervals in the clinical laboratory; Approved guideline-third edition." CLSI document EP28-A23c.
- Ismail, S. K. and J. R. Higgins (2011). "Hemostasis in pre-eclampsia." Semin Thromb Hemost **37**(2): 111-117.
- Juhan-Vague, I. and P. E. Morange (2003). "Very high TAFI antigen levels are associated with a lower risk of hard coronary events: the PRIME Study." J Thromb Haemost **1**(10): 2243-2244.
- Juhan-Vague, I., P. E. Morange, et al. (2002). "Plasma thrombin-activatable fibrinolysis inhibitor antigen concentration and genotype in relation to myocardial infarction in the north and south of Europe." Arterioscler Thromb Vasc Biol **22**(5): 867-873.
- Kaandorp, S., M. Goddijn, et al. (2010). "Aspirin plus heparin or aspirin alone in women with recurrent miscarriage." N Engl J Med **362**: 1586-1596.
- Kadir R, C. C., Bolton-Maggs P (2009). "Pregnancy and rare bleeding disorders." Haemophilia **15**(5): 990-1005.

- Kadir RA, L. C., Sabin CA, Pollard D, Economidies DL (1998). "Pregnancy in women with von Willebrand's Disease or factor XI deficiency." Br J Obstet Gynaecol **105**(3): 413-421.
- Karumanchi, S. A. and Y. Bdolah (2004). "Hypoxia and sFlt-1 in preeclampsia: the "chicken-and-egg" question." Endocrinology **145**(11): 4835-4837.
- Kendall, R. L. and K. A. Thomas (1993). "Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor." Proc Natl Acad Sci U S A **90**(22): 10705-10709.
- Kendall, R. L., G. Wang, et al. (1996). "Identification of a natural soluble form of the vascular endothelial growth factor receptor, FLT-1, and its heterodimerization with KDR." Biochem Biophys Res Commun **226**(2): 324-328.
- Khan, F., J. J. Belch, et al. (2005). "Changes in endothelial function precede the clinical disease in women in whom preeclampsia develops." Hypertension **46**(5): 1123-1128.
- Kikuchi Y, K. A. (2002). "A role for C5a in augmenting IgG-dependent histamine release from basophils in chronic urticaria." J Allergy Clin Immunol **109**: 114-118.
- Kingdom J, D. S. (2011). "Is heparin a placental anticoagulant in high risk pregnancies?" Blood **118**: 4780-4788.
- Kjellberg, U., N. E. Andersson, et al. (1999). "APC resistance and other haemostatic variables during pregnancy and puerperium." Thromb Haemost **81**(4): 527-531.
- Kleinrouweler C, W. M., Ris-Stalpers C, et al. (2012). "Accuracy for circulating placental growth factor, vascular endothelial growth factor, soluble fms like tyrosine kinase 1 and soluble endoglin in the prediction of pre-eclampsia: a systematic review and meta analysis." BJOG **119**: 778-787.
- Klement P, L. P., Bajzar L. (1999). "A novel approach to arterial thrombolysis." Blood **94**: 2735-2743.
- Klonoff-Cohen, H. S., D. A. Savitz, et al. (1989). "An epidemiologic study of contraception and preeclampsia." JAMA **262**(22): 3143-3147.
- Knight M, T. D., Kenyon S, Shakespeare J, Gray R, Kurinczul JJ (Eds) on behalf of the MBRACE-UK collaboration (2015). "Saving Lives, Improving mothers' care- Surveillance of maternal deaths in the UK 2011-2013 and lessons learned to inform maternity care from the UK and Ireland Confidential enquiries into Maternal deaths and morbidity 2009-2013 " Oxford: National Perinatal Epidemiology Unit, University of Oxford 2015
- Knol, H., N. Veeger, et al. (2009). "High thrombin activatable fibrinolysis inhibitor levels may protect against recurrent fetal loss." J Thromb Haemost **7**: 903-906.
- Koga, K., Y. Osuga, et al. (2010). "Elevated serum soluble fms-like tyrosine kinase 1 (sFlt1) level in women with hydatidiform mole." Fertil Steril **94**(1): 305-308.
- Kruithof, E. K., C. Tran-Thang, et al. (1987). "Fibrinolysis in pregnancy: a study of plasminogen activator inhibitors." Blood **69**(2): 460-466.
- Kuczynski, J., W. Uszynski, et al. (2002). "Tissue factor (TF) and tissue factor pathway inhibitor (TFPI) in the placenta and myometrium." Eur J Obstet Gynecol Reprod Biol **105**(1): 15-19.
- Kvehaugen AS, D. R., Ramstad HB, Troisi R, Fugelseth D, Staff AC. (2011). "Endothelial function and circulating biomarkers are disturbed in women and children after pre-eclampsia." Hypertension **58**: 63-69.
- Lafayette, R. A., M. Druzin, et al. (1998). "Nature of glomerular dysfunction in pre-eclampsia." Kidney Int **54**(4): 1240-1249.

- Lain, K. Y. and J. M. Roberts (2002). "Contemporary concepts of the pathogenesis and management of preeclampsia." JAMA **287**(24): 3183-3186.
- Lala, P. and C. Chakraborty (2003). "Factors regulating trophoblastic migration and invasiveness: possible derangements contributing to pre-eclampsia and fetal injury." Placenta **24**: 575-587.
- Lanir, N., A. Aharon, et al. (2003). "Haemostatic mechanisms in human placenta." Best Pract Res Clin Haematol **16**(2): 183-195.
- Laskin, C., K. Spitzer, et al. (2009). "Low molecular weight heparin and aspirin for recurrent pregnancy loss: results from the randomised controlled HepASA trial." J Rheumatol **36**: 279-287.
- Lawson, J. H., M. Kalafatis, et al. (1994). "A model for the tissue factor pathway to thrombin. I. An empirical study." J Biol Chem **269**(37): 23357-23366.
- Lawson, J. H. and K. G. Mann (1991). "Cooperative activation of human factor IX by the human extrinsic pathway of blood coagulation." J Biol Chem **266**(17): 11317-11327.
- Lefkowitz, J. B., S. H. Clarke, et al. (1996). "Comparison of protein S functional and antigenic assays in normal pregnancy." Am J Obstet Gynecol **175**(3 Pt 1): 657-660.
- Legnani, C., M. Bovara, et al. (2012). "Risk of early recurrent fetal loss and levels of thrombin activatable fibrinolysis inhibitor." Thromb Res **130**: 237-241.
- Leslie K, T. B., Papageorghiou (2011). "Early prediction and prevention of pre-eclampsia." Best Pract Res Clin Obstet Gynaecol **25**: 343-354.
- Levine, R. J., C. Lam, et al. (2006). "Soluble endoglin and other circulating antiangiogenic factors in preeclampsia." N Engl J Med **355**(10): 992-1005.
- Levine, R. J., S. E. Maynard, et al. (2004). "Circulating angiogenic factors and the risk of preeclampsia." N Engl J Med **350**(7): 672-683.
- Lie RT, R. S., Brunborg H, Gjessing HK, Lie-Neilsen E, Irgens LM (1998). "Fetal and maternal contributors to reisk of pre-eclampsia: a population based study." BMJ **316**: 1343-1347.
- Liu ZM, Z. S., Qin XJ, Cheng ZD, Lui MY, Zhang HM, Liu DX (2010). "Silencing of the C5a receptor gene with siRNA for protection from Gram-negative bacterial lipopolysaccharide- induced vascular permeability." Mol Immunol **47**: 1325-1333.
- Lockwood, C. J., G. Krikun, et al. (2000). "Progesterin-epidermal growth factor regulation of tissue factor expression during decidualization of human endometrial stromal cells." J Clin Endocrinol Metab **85**(1): 297-301.
- Lockwood, C. J., P. Matta, et al. (2006). "Regulation of monocyte chemoattractant protein-1 expression by tumor necrosis factor-alpha and interleukin-1beta in first trimester human decidual cells: implications for preeclampsia." Am J Pathol **168**(2): 445-452.
- Loskutoff, D. J. (1988). "Type 1 plasminogen activator inhibitor and its potential influence on thrombolytic therapy." Semin Thromb Hemost **14**(1): 100-109.
- Macfarlane, R. G. and R. Biggs (1948). "Fibrinolysis; its mechanism and significance." Blood **3**(10): 1167-1187.
- Macgillivray I, C. D. (1988). "Management of twin pregnancies In: Mac Gillivray, Campbell D, editors. Twinning and Twins." Chichester, UK. Wiley: 111-139.
- Maharaj, A. S., M. Saint-Geniez, et al. (2006). "Vascular endothelial growth factor localization in the adult." Am J Pathol **168**(2): 639-648.

- Maharaj, A. S., T. E. Walshe, et al. (2008). "VEGF and TGF-beta are required for the maintenance of the choroid plexus and ependyma." J Exp Med **205**(2): 491-501.
- Makris, A., C. Thornton, et al. (2007). "Uteroplacental ischemia results in proteinuric hypertension and elevated sFLT-1." Kidney Int **71**(10): 977-984.
- Mao, S. S., C. M. Cooper, et al. (1999). "Characterization of plasmin-mediated activation of plasma procarboxypeptidase B. Modulation by glycosaminoglycans." J Biol Chem **274**(49): 35046-35052.
- Martinez-Zamora, M. A., M. Creus, et al. (2010). "Thrombin activatable fibrinolysis inhibitor and clot lysis time in women with recurrent miscarriage associated with antiphospholipid syndrome." Fertil Steril **94**(6): 2437-2440.
- Martinez-Zamora, M. A., M. Creus, et al. (2011). "Reduced plasma fibrinolytic potential in patients with recurrent implantation failure after IVF and embryo transfer." Hum Reprod **26**(3): 510-516.
- Martinez-Zamora, M. A., D. Tassies, et al. (2010). "Clot lysis time and thrombin activatable fibrinolysis inhibitor in severe preeclampsia with or without associated antiphospholipid antibodies." J Reprod Immunol **86**(2): 133-140.
- Marx, P. F., T. H. Brondijk, et al. (2008). "Crystal structures of TAFI elucidate the inactivation mechanism of activated TAFI: a novel mechanism for enzyme autoregulation." Blood **112**(7): 2803-2809.
- Masini, S., C. Ticconi, et al. (2009). "Thrombin activatable fibrinolysis inhibitor polymorphisms and recurrent pregnancy loss." Fertil Steril **92**: 694-702.
- Matsuo, K., S. Kooshesh, et al. (2007). "Late postpartum eclampsia: report of two cases managed by uterine curettage and review of the literature." Am J Perinatol **24**(4): 257-266.
- Mattar, R., A. M. Amed, et al. (2004). "Preeclampsia and HIV infection." Eur J Obstet Gynecol Reprod Biol **117**(2): 240-241.
- Maynard, S. E., J. Y. Min, et al. (2003). "Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia." J Clin Invest **111**(5): 649-658.
- McDonald SD, M. A., Zhou Q, Yusuf S, Devereaux PJ. (2008). "Cardiovascular sequelae of pre-eclampsia/ eclampsia: a systematic review and meta-analysis." Am Heart J **156**: 918-930.
- McKeeman, G. C., J. E. Ardill, et al. (2004). "Soluble vascular endothelial growth factor receptor-1 (sFlt-1) is increased throughout gestation in patients who have preeclampsia develop." Am J Obstet Gynecol **191**(4): 1240-1246.
- Meijers, J. C., E. J. Oudijk, et al. (2000). "Reduced activity of TAFI (thrombin-activatable fibrinolysis inhibitor) in acute promyelocytic leukaemia." Br J Haematol **108**(3): 518-523.
- Mol, B. R. C. T. S. M. L. d. G. C. H. G. (2016). "Pre-eclampsia." Lancet **387**: 999-1011.
- Mollnes TE, B. O., Fung M, Fure H, Christiansen D, Bergseth G, Videm V, Lappégard KT, Kohl J, Lambris JD (2002). "Essential role of the C5a receptor in the e coli induced oxidative burst and phagocytosis revealed by a novel lepirudin based human whole blood model of inflammation " Blood **100**: 1869-1877.
- Mone F, M. F. (2014). "Low dose aspirin and calcium supplementation for the prevention of pre-eclampsia." TOG **16**: 245-250.
- Montaner, J., M. Ribo, et al. (2003). "Thrombin-activable fibrinolysis inhibitor levels in the acute phase of ischemic stroke." Stroke **34**(4): 1038-1040.

- Monte, S. (2011). "Biochemical markers for the prediction of pre-eclampsia: a review of the literature." *J Renal Med* **5**(3): 69-77.
- Mousa, H. A., C. Downey, et al. (2004). "Thrombin activatable fibrinolysis inhibitor and its fibrinolytic effect in normal pregnancy." *Thromb Haemost* **92**(5): 1025-1031.
- Mulligan MS, S. E., Beck-Schimmer B, Till GO, Freidl HP, Brauer RB, Hugli TE, Miyasaka M, Warner RL, Johnson KJ, Ward PA (1996). "Requirement and role of C5a in acute lung inflammatory injury in rats." *J Clin Invest* **98**: 503-512.
- Muto Y, S. K., Iida H et al (2009). "EF6265, a novel inhibitor of activated thrombin activatable fibrinolysis inhibitor, protects against sepsis induced organ dysfunction in rats." *Crit Care Med* **37**(5): 1744-1749.
- Muto Y, S. K., Sato E, Ishii H (2003). "Carboxypeptidase B inhibitors reduce tissue factor induced renal microthrombi in rats." *Eur J Pharmacol* **461**: 181-189.
- Myatt L, C. R., Roberts J, Spong C, Wapner R, Thorp J Jr, Mercer B, Peaceman A, Ramin S, Carpenter M, Sciscione A, Tolosa J, Saade G, Sorokin Y, Anderson G. (2012). "Can changes in angiogenic biomarkers between the first and second trimesters of pregnancy predict the development of pre-eclampsia in a low risk nulliparous patient population?" *BJOG* **120**: 1183-1191.
- Myles, T., T. Nishimura, et al. (2003). "Thrombin activatable fibrinolysis inhibitor, a potential regulator of vascular inflammation." *J Biol Chem* **278**: 1059-1067.
- Myles, T., T. Nishimura, et al. (2003). "Thrombin activatable fibrinolysis inhibitor, a potential regulator of vascular inflammation." *J Biol Chem* **278**(51): 51059-51067.
- Nagamatsu, T., T. Fujii, et al. (2004). "Cytotrophoblasts up-regulate soluble fms-like tyrosine kinase-1 expression under reduced oxygen: an implication for the placental vascular development and the pathophysiology of preeclampsia." *Endocrinology* **145**(11): 4838-4845.
- Nagashima M, W. M., Wang M, Zhao L, Light DR, Pagila R, Morser J, Verhallen P (2000). "An inhibitor of thrombin activatable fibrinolysis inhibitor potentiates tissue type plasminogen activator induced thrombolysis in a rabbit jugular vein thrombolysis model." *Thromb Res* **98**: 333-342.
- Nicolaides K, R. G., Hecher K. (2000). "Placental and fetal doppler." *Taylor and Francis Press*.
- Nilsson, I. M. and S. Kullander (1967). "Coagulation and fibrinolytic studies during pregnancy." *Acta Obstet Gynecol Scand* **46**(3): 273-285.
- Nishimura T, M. T., Piliponsky AM, Kao PN, Berry GJ, Leung LL (2007). "Thrombin activatable procarboxypeptidase B regulates activated complement C5a in vivo." *Blood* **109**: 1992-1997.
- Noraihan, M. N., P. Sharda, et al. (2005). "Report of 50 cases of eclampsia." *J Obstet Gynaecol Res* **31**(4): 302-309.
- North, R. A., C. Ferrier, et al. (1994). "Uterine artery Doppler flow velocity waveforms in the second trimester for the prediction of preeclampsia and fetal growth retardation." *Obstet Gynecol* **83**(3): 378-386.
- North RA, M. L., Dekker GA, et al (2011). "Clinical risk prediction for pre-eclampsia in nulliparous women: development of a model in international prospective cohort." *BMJ* **342**: d1875.
- O'Brien TE, R. J., Chan WS (2003). "Maternal body mass index and the risk of pre-eclampsia." *Epidemiology* **14**: 368-374.

- Oliver, J. J., D. J. Webb, et al. (2005). "Stimulated tissue plasminogen activator release as a marker of endothelial function in humans." Arterioscler Thromb Vasc Biol **25**(12): 2470-2479.
- Osmond, C., E. Kajantie, et al. (2007). "Infant growth and stroke in adult life: the Helsinki birth cohort study." Stroke **38**(2): 264-270.
- Paez MC, M. E., Diaz L, Shonfield Y, Serrano N, Anaya J (2013). "Lamin 1 in pre-eclampsia and systemic lupus erythematosus." Autoimmunity **46**(1): 14-20.
- Papageorghiou AT, Y. C., Cicero S, Bower S, Nicholaidies KH (2002). "Second trimester uterine artery doppler screening in unselected populations: a review." J Matern Fetal Neonatal Med **12**: 78-88.
- Papergeorghiou AT, Y. C., Erasmus IE, Cuckle HS, Nicholaides KH (2005). "Assessment of the risk of development of pre-eclampsia by maternal characteristics and uterine artery dopplers." BJOG **112**: 703-709.
- Parry, G. C. and N. Mackman (2000). "Mouse embryogenesis requires the tissue factor extracellular domain but not the cytoplasmic domain." J Clin Invest **105**(11): 1547-1554.
- Patel, T. V., J. A. Morgan, et al. (2008). "A preeclampsia-like syndrome characterized by reversible hypertension and proteinuria induced by the multitargeted kinase inhibitors sunitinib and sorafenib." J Natl Cancer Inst **100**(4): 282-284.
- Pedersen, B., T. Holscher, et al. (2005). "A balance between tissue factor and tissue factor pathway inhibitor is required for embryonic development and hemostasis in adult mice." Blood **105**(7): 2777-2782.
- Perry, K. G., Jr. and J. N. Martin, Jr. (1992). "Abnormal hemostasis and coagulopathy in preeclampsia and eclampsia." Clin Obstet Gynecol **35**(2): 338-350.
- Persson, B. L., P. Stenberg, et al. (1980). "Transamidating enzymes in maternal plasma and placenta in human pregnancies complicated by intrauterine growth retardation." J Dev Physiol **2**(1-2): 37-46.
- Poon LC, A. R., Lachmann R, Beta J, Nichoaides KH (2010). "Hypertensive disorders of pregnancy: screening by biophysical and biochemical markers at 11-13 weeks." Ultrasound Obstet Gynecol **35**: 662-670.
- Prick BW, B. D., Jansen AJ, et al (2015). "Determinants of health related quality of life in the postpartum period after obstetric complications." Eur J Obstet Gynecol Reprod Biol **185**: 88-95.
- Prisco, D., R. Paniccia, et al. (1994). "Euglobulin lysis time in fresh and stored samples." Am J Clin Pathol **102**(6): 794-796.
- Pruner, I., V. Djordjevic, et al. (2010). "+ 1040 C/T polymorphism in coding region of the thrombin-activatable fibrinolysis inhibitor gene and the risk of idiopathic recurrent fetal loss." Blood Coagul Fibrinolysis **21**: 679-682.
- Pryzdial, E. L. and K. G. Mann (1991). "The association of coagulation factor Xa and factor Va." J Biol Chem **266**(14): 8969-8977.
- Rafik Hamad, R., J. Curvers, et al. (2009). "Increased thrombin generation in women with a history of preeclampsia." Thromb Res **123**(4): 580-586.
- Rai, R. (2003). "Is miscarriage a coagulopathy?" Curr Opin Obstet Gynecol **15**: 265-268.
- Rai, R., E. G. D. Tuddenham, et al. (2003). "Thromboelastography whole-blood haemostasis and recurrent miscarriage." Hum Reprod **12**: 2540-2543.
- Rapaport, S. I. (1991). "Regulation of the tissue factor pathway." Ann N Y Acad Sci **614**: 51-62.
- Raymond D, P. E. (2011). "A critical review of early-onset and late-onset pre-eclampsia." Obstet Gynecol Surv **66**: 497-506.

- Redlitz, A., A. K. Tan, et al. (1995). "Plasma carboxypeptidases as regulators of the plasminogen system." J Clin Invest **96**(5): 2534-2538.
- Redman, C. W., G. P. Sacks, et al. (1999). "Preeclampsia: an excessive maternal inflammatory response to pregnancy." Am J Obstet Gynecol **180**(2 Pt 1): 499-506.
- Regan, L. and R. Rai (2002). "Thrombophilia and pregnancy loss." J Reprod Immunol **55**: 163-180.
- Reith, A., N. A. Booth, et al. (1993). "Plasminogen activator inhibitors (PAI-1 and PAI-2) in normal pregnancies, pre-eclampsia and hydatidiform mole." Br J Obstet Gynaecol **100**(4): 370-374.
- Rijken, D. C., M. Otter, et al. (1990). "Receptor-mediated endocytosis of tissue-type plasminogen activator (t-PA) by liver cells." Thromb Res Suppl **10**: 63-71.
- Robb, A. O., N. L. Mills, et al. (2009). "Acute endothelial tissue plasminogen activator release in pregnancy." J Thromb Haemost **7**(1): 138-142.
- Roberts, J. M. and H. S. Gammill (2005). "Preeclampsia: recent insights." Hypertension **46**(6): 1243-1249.
- Roberts, J. M. and K. Y. Lain (2002). "Recent Insights into the pathogenesis of pre-eclampsia." Placenta **23**(5): 359-372.
- Roberts, J. M., R. N. Taylor, et al. (1989). "Preeclampsia: an endothelial cell disorder." Am J Obstet Gynecol **161**(5): 1200-1204.
- Rodgers, G. M., R. N. Taylor, et al. (1988). "Preeclampsia is associated with a serum factor cytotoxic to human endothelial cells." Am J Obstet Gynecol **159**(4): 908-914.
- Romero, R., J. K. Nien, et al. (2008). "A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate." J Matern Fetal Neonatal Med **21**(1): 9-23.
- Romero R, N. J., Espinoza J, Todem D, Fu W, Chung H, Kusanovic JP, Gotsch F, Erez O, Maziki-Tovi S, Gomez R, Edwin S, Chaiworapongsa T, Levine RJ, Karumanchi SA. (2008). "A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endogulin and soluble vascular endothelial growth factor receptor -1) factors in normal pregnancy and patients destined to develop pre-eclampsia and deliver a small for gestational age neonate." J Matern Fetal Neonatal Med **21**(1): 9-23.
- Roos N, K. H., Sahlin L, Ekman_Ordeberg G, Falconer H, Stephansson O (2011). "Risk of adverse pregnancy outcomes in women with polycystic ovarian syndrome: population based cohort study." BMJ **343**: d6309.
- Rosenkranz, A., M. Hiden, et al. (2008). "Calibrated automated thrombin generation in normal uncomplicated pregnancy." Thromb Haemost **99**(2): 331-337.
- Rousseau, A., R. Favier, et al. (2009). "Elevated circulating soluble thrombomodulin activity, tissue factor activity and circulating procoagulant phospholipids: new and useful markers for pre-eclampsia?" Eur J Obstet Gynecol Reprod Biol **146**(1): 46-49.
- Salafia, C. M., J. C. Pezzullo, et al. (1998). "Clinical correlations of patterns of placental pathology in preterm pre-eclampsia." Placenta **19**(1): 67-72.
- Salomon, O., D. M. Steinberg, et al. (2003). "Inherited factor XI deficiency confers no protection against acute myocardial infarction." J Thromb Haemost **1**(4): 658-661.

- Sanchez-Luceros, A., S. S. Meschengieser, et al. (2003). "Factor VIII and von Willebrand factor changes during normal pregnancy and puerperium." Blood Coagul Fibrinolysis **14**(7): 647-651.
- Sandrim, V. C., A. C. Palei, et al. (2008). "Nitric oxide formation is inversely related to serum levels of antiangiogenic factors soluble fms-like tyrosine kinase-1 and soluble endogline in preeclampsia." Hypertension **52**(2): 402-407.
- Santamaria, A., A. Oliver, et al. (2003). "Risk of ischemic stroke associated with functional thrombin-activatable fibrinolysis inhibitor plasma levels." Stroke **34**(10): 2387-2391.
- Sattar N, B. A., Berry C, Shepard J, Greer IA, Packard CJ. (1997). "Lipoprotein subfraction concentrations in pre-eclampsia: pathogenic parallels to atherosclerosis." Obstet Gynecol **89**: 403-408.
- Schatteman, K. A., F. J. Goossens, et al. (2000). "Proteolytic activation of purified human procarboxypeptidase U." Clin Chim Acta **292**(1-2): 25-40.
- Scheid CR, W. R., Henson PM, Findlay SR (1983). "Direct effect of complement factor C5a on the contractile state of isolated smooth muscle." J Immunol **130**: 1997-1999.
- Schjetlein, R., G. Haugen, et al. (1997). "Markers of intravascular coagulation and fibrinolysis in preeclampsia: association with intrauterine growth retardation." Acta Obstet Gynecol Scand **76**(6): 541-546.
- Schneider, M., M. Boffa, et al. (2002). "Two naturally occurring variants of TAFI (Thr-325 and Ile-325) differ substantially with respect to thermal stability and antifibrinolytic activity of the enzyme." J Biol Chem **277**(2): 1021-1030.
- Schneider, M. and M. Nesheim (2003). "Reversible inhibitors of TAFIa can both promote and inhibit fibrinolysis." J Thromb Haemost **1**(1): 147-154.
- Schroeder, V., T. Chatterjee, et al. (2002). "Thrombin activatable fibrinolysis inhibitor (TAFI) levels in patients with coronary artery disease investigated by angiography." Thromb Haemost **88**(6): 1020-1025.
- Schroeder, V., N. Kucher, et al. (2003). "Role of thrombin activatable fibrinolysis inhibitor (TAFI) in patients with acute pulmonary embolism." J Thromb Haemost **1**(3): 492-493.
- Sebire, N., M. Backos, et al. (2003). "Placental pathology, antiphospholipid antibodies, and pregnancy outcome in recurrent miscarriage patients." Obstet Gynecol **101**: 258-263.
- Shembrey, M. A. and A. D. Noble (1995). "An instructive case of abdominal pregnancy." Aust N Z J Obstet Gynaecol **35**(2): 220-221.
- Sheppard, B. L. and J. Bonnar (1999). "Uteroplacental hemostasis in intrauterine fetal growth retardation." Semin Thromb Hemost **25**(5): 443-446.
- Shinohara, T., C. Sakurada, et al. (1994). "Pro-carboxypeptidase R cleaves bradykinin following activation." Int Arch Allergy Immunol **103**(4): 400-404.
- Sibai, B., G. Dekker, et al. (2005). "Pre-eclampsia." Lancet **365**(9461): 785-799.
- Sibai, B. M. (2005). "Diagnosis, prevention, and management of eclampsia." Obstet Gynecol **105**(2): 402-410.
- Sibai, B. M., M. K. Ramadan, et al. (1993). "Maternal morbidity and mortality in 442 pregnancies with hemolysis, elevated liver enzymes, and low platelets (HELLP syndrome)." Am J Obstet Gynecol **169**(4): 1000-1006.
- Siemons JM, B. L. (1917). "The uric acid content of maternal and fetal blood." J Biol Chem **32**: 63-67.
- Silveira, A., K. Schatteman, et al. (2000). "Plasma procarboxypeptidase U in men with symptomatic coronary artery disease." Thromb Haemost **84**(3): 364-368.

- Skidgel RA, E. E. (2007). "Structure and function of human carboxypeptidase N, the anaphylatoxin inactivator " Int Immunopharmacol **7**: 1888-1899.
- Sotiriadis, A., A. Makrigrannakis, et al. (2007). "Fibrinolytic defects and recurrent miscarriage." Obstet Gynecol **109**: 1146-1155.
- Spargo, B., C. C. Mc, et al. (1959). "Glomerular capillary endotheliosis in toxemia of pregnancy." Arch Pathol **68**: 593-599.
- Stern, C. and L. Chamley (2006). "Antiphospholipid antibodies and coagulation defects in women with implantation failure after IVF and miscarriage." Reprod Biomed Online **13**: 29-37.
- Stirling, Y., L. Woolf, et al. (1984). "Haemostasis in normal pregnancy." Thromb Haemost **52**(2): 176-182.
- Strand KM, H. R., Iversen AC et al (2013). "Mediators of the association between pre-eclampsia and cerebral palsy: population based cohort study." BMJ **347**: f4089.
- Szecs PB, J. M., Klajnbard A, Andersen MR, Colov NP, Stender S. (2010). "Haemostatic reference intervals in pregnancy." Thromb Haemos **103**: 718-727.
- Tabesh M, S.-A. A., Y+Tabesh M, Esmailzadeh A. (2013). "Maternal vitamin D status and risk of pre-eclampsia: a systematic review and meta-analysis." J Clin Endocrinol Metab **98**(8): 3165-3173.
- Tanjung, M. T., H. D. Siddik, et al. (2005). "Coagulation and fibrinolysis in preeclampsia and neonates." Clin Appl Thromb Hemost **11**(4): 467-473.
- Thangaratinam S, G. I., Meah N, Usman S, Ismail KM, Khan KS and the TIPPS (Tests in the Prediction of Pre-eclampsia Severity) review group. (2011). "How accurate are maternal symptoms in predicting impending complications in women with pre-eclampsia? A systematic review and meta- analysis." Acta Obstet Gynecol Scand **90**: 564-573.
- Thangaratinam S, I. K., Sharp S, Coomarasamy A, Khan K. (2006). "Accuracy of serum uric acid in predicting complications of pre-eclampsia: a systematic review." BJOG **113**: 369-378.
- Thornton, C. A. and J. Bonnar (1977). "Factor VIII-related antigen and factor VIII coagulant activity in normal and pre-eclamptic pregnancy." Br J Obstet Gynaecol **84**(12): 919-923.
- Tranquilli AL, D. G., Magee L, et al (2014). "The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP." Pregnancy Hypertens **4**: 97-104.
- Tubbergen, P., A. M. Lachmeijer, et al. (1999). "Change in paternity: a risk factor for preeclampsia in multiparous women?" J Reprod Immunol **45**(1): 81-88.
- Tuffnell DJ, J. D., Lindow SW et al (2005). "Outcomes of severe pre-eclampsia/ eclampsia in Yorkshire 1999-2003." BJOG **112**(7): 875-880.
- Uchikova, E. H. and Ledjev, II (2005). "Changes in haemostasis during normal pregnancy." Eur J Obstet Gynecol Reprod Biol **119**(2): 185-188.
- Valnikova Z, T. I., Potempa J, Enghild JJ (2007). "Thrombin activatable fibrinolysis inhibitor (TAFI) zymogen is an active carboxypeptidase." J Biol Chem **279**: 3066-3076.
- van Tilburg, N. H., F. R. Rosendaal, et al. (2000). "Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis." Blood **95**(9): 2855-2859.
- van Wersch, J. W. and J. M. Ubachs (1991). "Blood coagulation and fibrinolysis during normal pregnancy." Eur J Clin Chem Clin Biochem **29**(1): 45-50.

- Vanhoof, G., J. Wauters, et al. (1996). "The gene for human carboxypeptidase U (CPU)--a proposed novel regulator of plasminogen activation--maps to 13q14.11." Genomics **38**(3): 454-455.
- Vatten LJ, S. R. (2004). "Is pre-eclampsia more than one disease." BJOG **111**: 298-302.
- Veerbeek JH, H. W., Breimer AY, et al. (2015). "Cardiovascular disease risk factors after early onset pre-eclampsia, late onset pre-eclampsia and pregnancy induced hypertension." Hypertension **65**: 600-606.
- Vellanki, K. (2013). "Pregnancy in chronic renal disease." Advances in Chronic Kidney disease **20**(3): 223-228.
- Venkatesha, S., M. Toporsian, et al. (2006). "Soluble endoglin contributes to the pathogenesis of preeclampsia." Nat Med **12**(6): 642-649.
- von Dadelszen P, P. B., Li J, et al, and the PIERS Study Group (2011). "Prediction of adverse maternal outcomes in pre-eclampsia: development and validation of the fullPIERS model." Lancet **377**: 219-227.
- Walker JB, B. T., Mackova M, Lambkin GR, Mitchell L, Bajzar L (2008). "Proteolytic cleavage of carboxypeptidase N markedly increases its antifibrinolytic activity " Thromb Haemost **6**: 848-855.
- Wallukat, G., V. Homuth, et al. (1999). "Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor." J Clin Invest **103**(7): 945-952.
- Wang, J. X., A. M. Knottnerus, et al. (2002). "Surgically obtained sperm, and risk of gestational hypertension and pre-eclampsia." Lancet **359**(9307): 673-674.
- Wang YX, d. C. V., Vincelette J, Zhao L, Nagashima M, Kawai K, Yuan S, Emayan K, Islam I, Hosoya j, Sullivan ME, Dole WP, Morser J, Buckham BO, Vergona R (2007). "A novel inhibitor of activated thrombin activated fibrinolysis inhibitor (TAFIa) - part II: enhancement of both exogenous and endogenous fibrinolysis in animal models of thrombosis." Thromb Haemost **97**: 54-61.
- Warren BL, E. A., Singer P et al (2001). "Caring for the critically ill patient. High dose antithrombin III in severe epsis: a randomised controlled trial." JAMA **286**: 1869-1878.
- Watanabe, R., H. Wada, et al. (2001). "Activity and antigen levels of thrombin-activatable fibrinolysis inhibitor in plasma of patients with disseminated intravascular coagulation." Thromb Res **104**(1): 1-6.
- Watanabe, T., H. Minakami, et al. (2004). "Changes in activity of plasma thrombin activatable fibrinolysis inhibitor in pregnancy." Gynecol Obstet Invest **58**(1): 19-21.
- Waterstone M, B. S., Wolfe C (2001). "Incidence and predictors of severe obstetric morbidity: a case control study." BMJ **322**(7294): 1089-1093.
- Wathen, K. A., E. Tuutti, et al. (2006). "Maternal serum-soluble vascular endothelial growth factor receptor-1 in early pregnancy ending in preeclampsia or intrauterine growth retardation." J Clin Endocrinol Metab **91**(1): 180-184.
- Weiner, A. E., D. E. Reid, et al. (1949). "The Hemostatic Activity of Amniotic Fluid." Science **110**(2851): 190-191.
- Weisel, J. W., C. W. Francis, et al. (1993). "Determination of the topology of factor XIIIa-induced fibrin gamma-chain cross-links by electron microscopy of ligated fragments." J Biol Chem **268**(35): 26618-26624.

- Wickstrom, K., G. Edelstam, et al. (2004). "Reference intervals for plasma levels of fibronectin, von Willebrand factor, free protein S and antithrombin during third-trimester pregnancy." Scand J Clin Lab Invest **64**(1): 31-40.
- Willemse, J. L. and D. F. Hendriks (2006). "Measurement of procarboxypeptidase U (TAFI) in human plasma: a laboratory challenge." Clin Chem **52**(1): 30-36.
- Willemse, J. L., E. Heylen, et al. (2009). "Carboxypeptidase U (TAFIa): a new drug target for fibrinolytic therapy?" J Thromb Haemost **7**(12): 1962-1971.
- Wright D, A. R., Syngelaki A, Poon L, Nicholaides K. (2012). "A competing risks model in early screening for pre-eclampsia." Fetal Diagn Ther **32**: 171-178.
- Wright, J. G., P. Cooper, et al. (1988). "Fibrinolysis during normal human pregnancy: complex inter-relationships between plasma levels of tissue plasminogen activator and inhibitors and the euglobulin clot lysis time." Br J Haematol **69**(2): 253-258.
- Xia, Y., H. Wen, et al. (2003). "Maternal autoantibodies from preeclamptic patients activate angiotensin receptors on human trophoblast cells." J Soc Gynecol Investig **10**(2): 82-93.
- Young, B. C., R. J. Levine, et al. (2010). "Pathogenesis of preeclampsia." Annu Rev Pathol **5**: 173-192.
- Zhang, Y., Y. Hu, et al. (2008). "Thrombin activatable fibrinolysis inhibitor in preeclampsia and gestational hypertension throughout the gestation." J Huazhong Univ Sci Technolog Med Sci **28**(2): 140-143.
- Zhao, L., J. Morser, et al. (1998). "Identification and characterization of two thrombin-activatable fibrinolysis inhibitor isoforms." Thromb Haemost **80**(6): 949-955.
- Zhou, C. C., Y. Zhang, et al. (2008). "Angiotensin receptor agonistic autoantibodies induce pre-eclampsia in pregnant mice." Nat Med **14**(8): 855-862.
- Zhou, Y., C. H. Damsky, et al. (1997). "Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome?" J Clin Invest **99**(9): 2152-2164.
- Zhou, Y., S. J. Fisher, et al. (1997). "Human cytotrophoblasts adopt a vascular phenotype as they differentiate. A strategy for successful endovascular invasion?" J Clin Invest **99**(9): 2139-2151.
- Zhou, Y., M. McMaster, et al. (2002). "Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome." Am J Pathol **160**(4): 1405-1423.