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Full Length Article

Changes in laboratory markers of thrombotic risk early in the first trimester of pregnancy may be linked to an increase in estradiol and progesterone

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ABSTRACT

Background: Pregnant women are at increased risk of venous thrombosis compared to non-pregnant women. Epidemiological and laboratory data suggest that hypercoagulability begins in the first trimester but it is unknown exactly how early in pregnancy this develops. The mechanisms that result in a prothrombotic state may involve oestrogens and progestogens.

Methods: Plasma samples were taken prior to conception and five times in early pregnancy, up to Day 59 gestation, from 22 women undergoing natural cycle in vitro fertilization, who subsequently gave birth at term following a normal pregnancy.

Thrombin generation, free Protein S, Ddimer, Fibrinogen, factor VIII, estradiol and progesterone were measured. To counter inter-individual variability, the change in laboratory measurements between the pre-pregnant and pregnant state were measured over time.

Results: Peak thrombin, Endogenous Thrombin Potential, Velocity Index and fibrinogen significantly increased, and free Protein S significantly decreased, from pre-pregnancy levels, by 32 days gestation. Ddimer and VIII significantly increased from pre-pregnancy levels by 59 days gestation. Estradiol significantly increased by Day 32 gestation with a non-significant increase of 67% by Day 24 gestation. Progesterone significantly increased by Day 32 gestation. Almost all laboratory markers of thrombosis correlated significantly with estradiol and progesterone.

Conclusion: Our work is the first to demonstrate that the prothrombotic state develops very early in the first trimester. Laboratory markers of hypercoagulability correlate significantly with estradiol and progesterone suggesting these are linked to the prothrombotic state of pregnancy. Clinicians should consider commencing thromboprophylaxis early in the first trimester in women at high thrombotic risk.

1. Introduction

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

In a number of studies a significant proportion of antenatal venous thromboembolism (VTE) is reported to occur in the first trimester, with reported figures ranging between 11 and 21% of all antenatal VTE [4–6]. One study that particularly assessed the proportion of VTE occurring in each trimester reported that 44% of antenatal VTE occurred in the first trimester [2]. Furthermore, data from the UK maternal mortality database (MBRRACE-UK) indicates that 50% of antenatal deaths occur in the first trimester [7]. Finally, studies sometimes do not include women prior to 13 weeks gestation or exclude women with pregnancy losses (spontaneous, therapeutic or as a result of ectopic pregnancy) [4,5]. As a result of this and given that most pregnancy losses occur in the first trimester, the risk of VTE in the first trimester is likely significantly underestimated.

There are some well recognised changes in coagulation factor concentrations that occur during pregnancy. The concentration of Protein S, both total and free (the latter being the active form), decreases during pregnancy, resulting in a decrease in the downregulation of activated factors V and VIII [8–12]. An increase in factor VIII also occurs, likely

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secondary to both reduced Protein S and increased VWF levels, the latter extending the half-life of FVIII [8,9,12]. Fibrinogen, the precursor to fibrin, the main constituent of a thrombus, is increased during pregnancy [9]. Perhaps to counteract this increase in the plasma concentration of fibrinogen, there is increased fibrin turnover resulting in an increase in fibrin degradation products, such as ddimer [13–17]. Despite these characterizations, the time at which these changes first occur during pregnancy is unknown.

Thrombin generation, a global coagulation assay, has been demonstrated to more accurately reflect the thrombotic phenotype than measuring individual parameters of the coagulation cascade [18–22]. Thrombin is pivotal to the coagulation cascade; it is the enzyme responsible for converting fibrinogen to fibrin, the step resulting in clot formation. Thrombin also has numerous positive and negative feedback roles across the coagulation cascade, thus making it central to this process. It is therefore reasonable to expect that an assessment of an individual's ability to generate thrombin will provide an accurate evaluation of an individual's potential to coagulate.

Studies assessing thrombin generation during pregnancy have been undertaken but have had various limitations in their findings [21,23–25]. Rarely has thrombin generation been assessed earlier than 10 weeks of gestation and most studies have not obtained pre-pregnancy samples from the same women, preventing an account to be taken of the known significant inter-individual variability in thrombin generation [21,25–30]. Other potential confounders such as the use of low molecular weight heparin (LMWH) have also affected data [28,29].

We have recently demonstrated that thrombin generation, using any of the four most recognised assay conditions, increases from approximately the 5th gestational week [31]. This data was obtained from a cohort of women undergoing natural cycle in vitro fertilization, as such women provide the most practical method of obtaining accurately timed peri–conceptual and early pregnancy samples and are the best physiological representation of a 'normal' pregnancy, outside a free living population. These women subsequently gave birth at term following a normal pregnancy. This was the first study to assess thrombin generation during the first few weeks of pregnancy, use pre pregnancy samples from the same women as internal comparators, use internal quality controls and avoid confounders such as low molecular weight heparin (LMWH). We therefore believe that this is the most valid and accurate measurement of thrombin generation in early pregnancy to date.

The mechanism by which a prothrombotic state develops in pregnancy may involve estrogens and progestogens, whose levels are known to change during pregnancy. It is hypothesised that the prothrombotic state may develop secondary to increased levels of estradiol, this argument being strengthened by the increased risk of venous thrombosis and similar alterations of coagulation parameters in women exposed to exogenous estrogens contained within the combined oral contraceptive pill (COCP) and hormone replacement therapy (HRT) [22,32–34]. During pregnancy, estradiol production increases significantly, initially through secretion from the corpus luteum and towards the end of the first trimester, this role is taken over by the placenta [35]. Estrogen response elements (ERE) have been demonstrated upstream of many genes encoding for coagulation factors in the liver and exposure to estradiol affects coagulation factor transcription [36–40]. Taken together, these data make estradiol a prime candidate to investigate as a potential cause of the prothrombotic changes detected in the plasma of pregnant women. Progestogens also are linked to thrombosis risk, albeit this being much weaker than the link with estrogens. This link is mainly via epidemiological data from women exposed to exogenous progestogens in the COCP and HRT [41–46].

In this study we assessed the same cohort of women undergoing natural cycle in vitro fertilization, as previously described, to determine whether, alongside thrombin generation, levels of well recognised laboratory features of the prothrombotic state of pregnancy e.g. FVIII, ddimer, fibrinogen and protein S change at a similar stage of pregnancy as thrombin generation. We investigated whether these coagulation factors correlate with thrombin generation when using what is considered to be the most 'sensitive' thrombin generation assay method i.e. 1 pM TF with thrombomodulin where both the intrinsic, extrinsic and activated Protein C (APC) pathways are included in the assessment. To establish a potential mechanism for any change in thrombotic markers early in pregnancy, levels of estradiol and progesterone were also measured over the same time period.

Our aim was to demonstrate how early in pregnancy laboratory markers of the prothrombotic state begin to develop, to establish a potential mechanism for these changes and in turn, guide clinicians as to how early preventative measures should be taken in pregnant women at high risk of thrombosis.

2. Materials and methods

2.1. Participants

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

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

2.2. Sample collection

Venous blood was collected into 0.109 M sodium citrate or 0.369 M tripotassium EDTA and centrifuged at 3000 rpm for 15 min at 4 °C within 2 h of collection. Citrated plasma was spun a second time in a microfuge at 13,000 rpm for 4 min to obtain platelet poor plasma (PPP). All plasma was stored at −80 °C.

Within the study, Study Day −3 was considered to be equivalent to Day 14 from last menstrual period (LMP) in a naturally occurring pregnancy (i.e. 2 weeks gestation assuming a 28 day menstrual cycle). Pre-pregnancy samples were taken approximately at the time of the lutenizing hormone surge (Study Day −3) and at the time of frozen embryo transfer (Study Day 0). Up to a further 5 samples were then taken from the same women very early in gestation, at Study Day +4, +7, +15, +26 and + 42, with Study Day +42 being equivalent to estimated Day 59 gestation (Fig. 1).

2.3. Laboratory investigations

All measurements were carried out at Glasgow Royal Infirmary, with samples from each woman assessed concurrently to minimise the effect of inter-assay variability. Commercial reagents were used according to the manufacturers' instructions.

2.4. Thrombin generation

Thrombin generation (TG) was measured using the Calibrated Automated Thromobogram method (CAT, Thrombinoscope, BV, Maastricht, Netherlands) as described previously [31]. Briefly, 80 µl of platelet poor plasma were mixed with 20 µl PPPLow (1 pM tissue factor [TF]), in the presence of 0.4 nM thrombomodulin (TM) and 20 µl FluCa solution, where the optimal concentration of TM used was determined as described [47]. TG reagents were purchased from Diagnostica Stago (Asnières, France), TM was donated by Cardiovascular Research

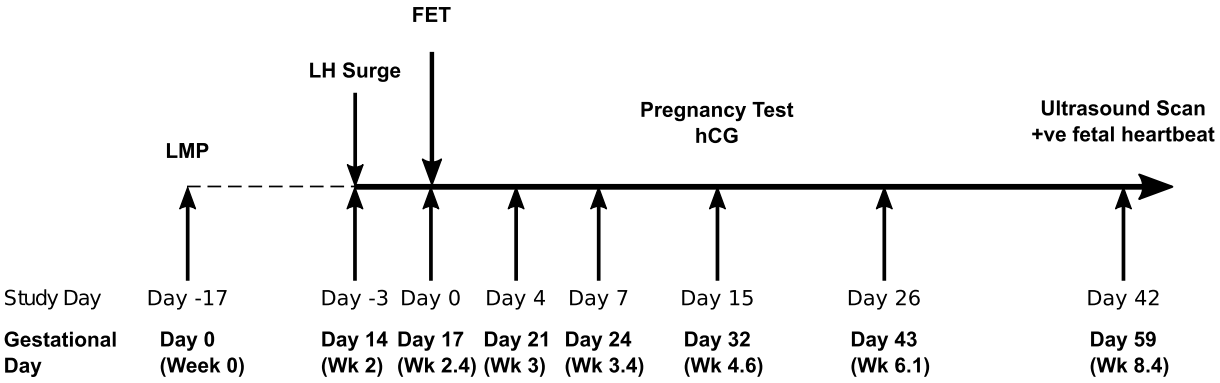

Fig. 1. Sample time points and equivalent gestational period.
LH: lutenizing hormone; FET: frozen embryo transfer; hCG: human chorionic gonadotrophin.

Institute Maastricht (CARIM), The Netherlands and TGT Reference plasma (TGT-RP) was a gift from NIBSC (Potters Bar, Hertfordshire, UK). TG parameters measured were; time to the first thrombin production (lag time), the maximum concentration of thrombin generated (Peak Thrombin), the slope between first thrombin production and the time to achieve Peak Thrombin (Velocity Index), time to reach peak height (ttPeak), time at which thrombin generation ceases (start tail) and the area under the curve (Endogenous Thrombin Potential, ETP).

2.5. Estradiol and progesterone

Estradiol and progesterone levels were determined with the commercially available chemiluminescent microparticle immunoassay kits; Architect Estradiol 7K72 and Architect Progesterone 7K77, using the architect iSR 2000 machine (Abbott Diagnostics).

2.6. Other laboratory measurements

For all other coagulation assays, reagents were purchased from Instrumentation Laboratory and the automated analyser ACL TOP 700 was used. Protein S and D-Dimer were measured by automated latex enhanced immunoassays using Free Protein S and D-Dimer HS Kits. Fibrinogen levels were measured by the Clauss method, using QFA-Fib, and FVIII was measured by a one-stage clotting assay, with Factor VIII deficient plasma and Synthasil (APTT Reagent).

2.7. Statistical analysis

Data are expressed as percentage change from baseline (pre-pregnancy) and reported as mean ± 95% confidence interval (C.I.) for parametric and median ± interquartile range for non-parametric data. Statistical analyses were performed using GraphPad Prism. To compare results between study time points for each laboratory measurement, for parametric data ANOVA followed by Dunnett's multiple comparison tests were used, and for non-parametric data a Kruskal-Wallis test was used. Spearman's rank correlation was used to assess the association between laboratory measurements. Statistical significance was given a P-value of < 0.05.

3. Results

196 women were recruited, with 36 successful first attempt pregnancies, of which 22 women gave birth at term with no complications occurring during the antenatal period. Demographic data for these 22 women is shown in Table S1, including the reason for IVF.

3.1. Effect of pregnancy on thrombin generation

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

On analysis of the mean raw data at study time points, there was a significant increase at Study Day +26 (approximately Day 43 gestation) from baseline (average of Study Days -3 & 0 [pre-pregnancy]) for mean peak thrombin and mean ETP. This increase persisted to Study Day +42 (approximately gestational day 59). To minimise the effect of inter-individual variability, the mean change in thrombin generation over time was also determined i.e. change between the pre-pregnant and pregnant state *per* individual. There was a significant change from baseline (pre-pregnancy) at Study Day +15 (approximately Day 32 gestation) for peak thrombin, ETP and VI. This increase persisted into the 6th and 8th gestational week (Study Days +26 & +42 respectively). Table 1 summarises all mean raw data generated for ETP, peak and VI. Fig. 2 illustrates the data for mean percentage change from baseline. The women's data normalised to results from the standard plasma (TGT-RP) produced identical/very similar statistically significant outcomes (data not shown).

Table 1
Thrombin generation mean raw data for pre-pregnancy and early pregnancy.

	Day −3 (n = 21)	Day 0 (n = 22)	Day +4 (n = 13)	Day +7 (n = 12)	Day +15 (n = 22)	Day +26 (n = 16)	Day +42 (n = 19)
ETP (nM.min)	894 (768, 1019)	760 (621, 898)	923 (717, 1130)	1007 (839, 1175)	1019 (877, 1161)	[b]1186 (1019, 1352)	[c]1278 (1144, 1413)
Peak thrombin (nM)	167 (137, 197)	136 (107, 165)	172 (127, 217)	186 (151, 222)	190 (161, 218)	[a]211 (177, 246)	[b]230 (201, 260)
Velocity Index	52 (39, 64)	41 (30, 52)	53 (37, 70)	59 (43, 76)	61 (49, 72)	66 (51, 80)	[a]72 (59, 86)

Mean raw data for ETP, peak and Velocity Index between pre-pregnancy and early pregnancy. Pre-pregnancy values are an average of Study Day −3 and Study Day 0. Each box represents the mean value with 95% confidence intervals in brackets. (a) represents $p < 0.01$; (b) represents $p < 0.001$; (c) represents $p < 0.0001$. P represents the comparison between the mean at baseline (average of Day −3 and Day 0) and the mean at individual time points during early pregnancy. p values are based on ANOVA for continuous variables. n = number of samples available for analysis at that time point. n/a = not applicable, ETP: endogenous thrombin potential.

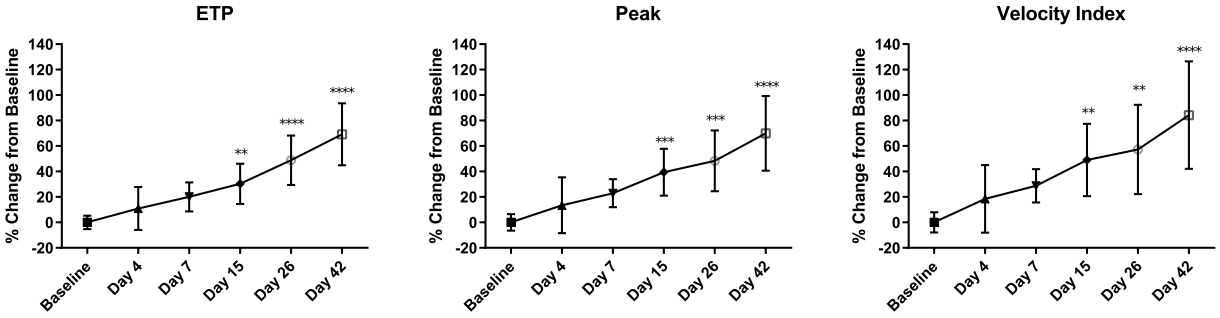

Fig. 2. Mean percentage change from baseline in early pregnancy in thrombin generation at 1 pM TF + TM.
Mean data ± 95% C.I. for percentage change from baseline under 1 pM TF + TM assay conditions. Baseline is the mean of Study Day −3 and Study Day 0. Change from baseline is the change in a thrombin generation parameter between baseline and a given time point in early pregnancy e.g. Day +15 compared to baseline. **represents $p < 0.01$; ***represents $p < 0.001$; ****represents $p < 0.0001$. TF: tissue factor; TM: thrombomodulin; ETP: endogenous thrombin potential.

3.2. Other laboratory measurements

Protein S demonstrated a significant reduction from baseline by Study Day +15 (approximately Day 32 gestation) ($p < 0.01$), and this decrease persisted to gestational Day 59. Similarly, fibrinogen changed significantly by Study Day +15 (approximately Day 32 gestation) ($p < 0.01$), with an increase from baseline, and this persisted to gestational Day 59. D-dimer and FVIII also increased significantly ($p < 0.0001$) by Study Day +42 (approximately Day 59 gestation) as shown in Table 2 and Fig. 3.

3.3. Estradiol and progesterone

Estradiol significantly increased by Study Day +15 (approximately Day 32 gestation) with a non-significant increase of 67% by Study Day +7 (Fig. 3). Progesterone significantly increased by Study Day +15 (approximately Day 32 gestation).

3.4. Correlation between thrombin generation parameters, other coagulation factors and estradiol and progesterone

The thrombin generation parameters ETP, Peak thrombin and Velocity Index, correlated significantly with estradiol (Fig. 4) and all other laboratory markers measured (Table 3). All the laboratory markers measured correlated significantly with Estradiol and with each other with the exception of Progesterone, where correlation with FVIII was not significant (Table 4).

4. Discussion

We have demonstrated for the first time the very early stage at which laboratory markers of coagulability change in pregnancy. Protein S, fibrinogen, ddimer and FVIII have previously been known to be affected by pregnancy but this is the first work to show how early in pregnancy these markers change. Furthermore, thrombin generation has also been shown to increase during pregnancy and our previous recent work demonstrated that thrombin generation, under all of the well recognised assay conditions, increases early in pregnancy, around the 5th week of gestation [31].

Our current work has demonstrated that the procoagulant state of pregnancy begins within the first few weeks of gestation, with a decrease in Protein S alongside a concurrent increase in fibrinogen, ddimer, FVIII and thrombin generation. All of the changes in the coagulation factors occurred at a similar time and their levels correlated, suggesting a similar mechanism for all of them changing to a more thrombotic profile. The correlation of thrombin generation parameters with all of these coagulation factors is further evidence that thrombin generation can give a global overview of the coagulation status of a patient.

The development of a prothrombotic profile appears to start at the beginning of the 5th gestational week as there is clearly a significant change in thrombin generation, Protein S and ddimer at this time. However, these changes may be occurring even earlier as the quantity of plasma available at Study Day +7 was small as not all women had sufficient plasma samples available for analysis at each time point. For example, Protein S and fibrinogen had changed by approximately 20% and ddimer by 50% from baseline to Study Day +7 (approximately 24 days gestation) despite plasma only being available from 4 women at that time. With plasma samples available from more women at this time, an earlier significant change may have been seen.

Estradiol increased significantly from baseline at approximately 4 weeks gestation, at the same time as the changes seen in the coagulation factors. However, for the reasons given above, that not every women had sufficient plasma samples available for analysis at every time point, it is highly likely that these changes occurred at an earlier gestation. From approximately 24 days gestation, the increase in

Table 2
Median raw data for Estradiol, Progesterone, Protein S, D-Dimer, Fibrinogen and FVIII.

	Baseline (n = 22)	Day +4 (n = 13)	Day +7 (n = 12)	Day +15 (n = 22)	Day +26 (n = 16)	Day +42 (n = 19)
Estradiol (pmol/l)	374 (428)	453 (184)	491 (274)	[b]650 (454)	[d]1362 (805)	[d]3194 (2645)
Progesterone (nmol/l)	1	n/t	n/t	[d]69.35 (71.1)	[d]69.2 (50.3)	[d]58.5 (29.6)
Protein S (%)	109.25 (17.3)	109 (18.1)	102.05 (16.4)	92.05 (4.5)	[c]71.3 (24.9)	[d]48.8 (24.6)
D-Dimer (ng/ml)	46.5 (60)	[a]121 (59)	73.5 (12)	88 (51.3)	109 (73)	[b]131 (67)
Fibrinogen (g/l)	2.6 (2.3)	2.8 (0.9)	2.73 (0.4)	3.36 (0.6)	3.16 (0.4)	[b]3.59 (0.6)
FVIII (%)	95.9 (26.5)	95.55 (16.9)	88.57 (10.8)	102.8 (27.7)	95.6 (22.7)	[a]125.5 (52.45)

Median raw data between pre-pregnancy and early pregnancy for Estradiol, Progesterone, Protein S, D-Dimer, Fibrinogen and FVIII. Pre-pregnancy values are an average of Study Day −3 and Study Day 0. Each box represents the median value with the interquartile range in brackets. n/a = not applicable, n/t = not tested, (a) represents $p < 0.05$; (b) represents $p < 0.01$; (c) represents $p < 0.001$; (d) represents $p < 0.0001$. P represents the comparison between the median at baseline (average of Day −3 and Day 0) and the median at individual time points during early pregnancy. p values are based on the Kruskal-Wallis test for continuous variables. n = number of samples available for analysis at that time point.

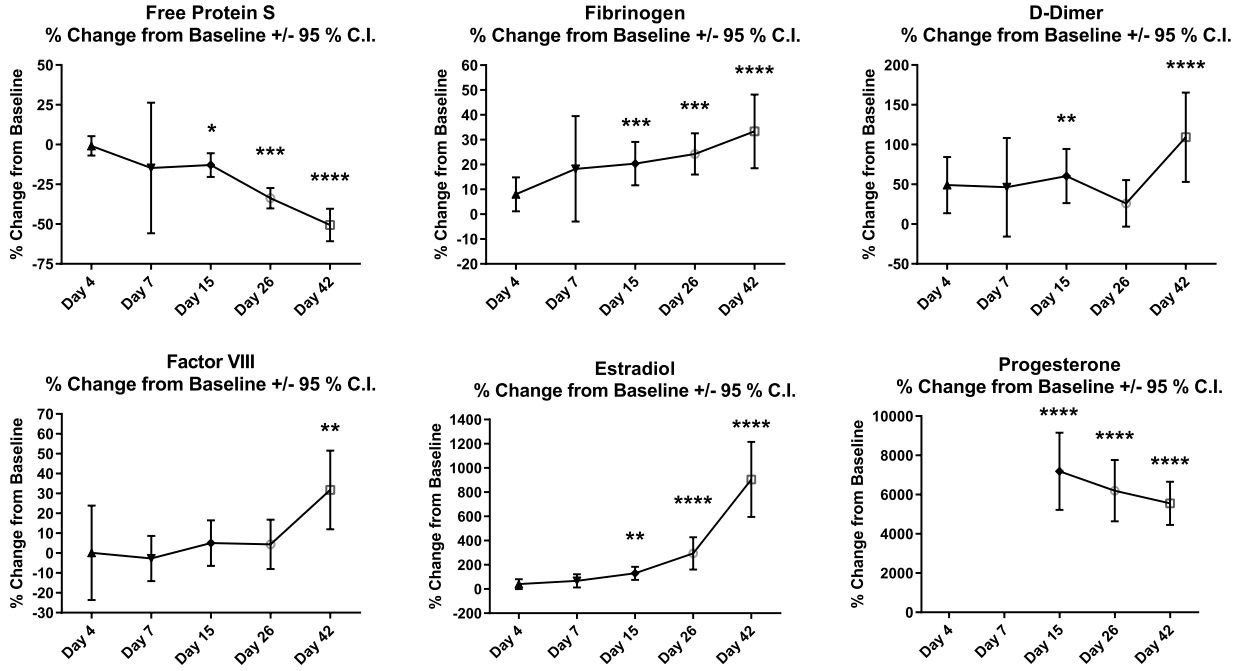

Fig. 3. Mean percentage change from baseline in early pregnancy in Estradiol, Progesterone, Protein S, D-Dimer, Fibrinogen and FVIII.
Mean data ± 95% C.I. for percentage change from baseline. Baseline is the mean of Study Day −3 and Study Day 0. Change from baseline is the change in a thrombin generation parameter between baseline and a given time point in early pregnancy e.g. Day +15 compared to baseline. * represents $p < 0.05$, **represents $p < 0.01$; ***represents $p < 0.001$; ****represents $p < 0.0001$.

estradiol from the pre pregnancy state was 67%, a substantial rise, highly suggestive that with greater numbers, it would have been possible to demonstrate that estradiol increases significantly enough to subsequently have an impact on coagulation factor gene expression.

It is the data regarding correlation between all the coagulation factors, estradiol and progesterone which provides the strongest evidence for a relationship between these proteins. All the significantly increased thrombin generation markers correlated significantly with all the other coagulation factors and the individual coagulation factors correlated with each other. Perhaps most interestingly there was a strong correlation between estradiol and all the coagulation parameters, including thrombin generation, suggesting a relationship between the increased production of estradiol during early pregnancy and changes in coagulation protein levels. Estrogen response elements (ERE) have been demonstrated upstream of a number of coagulation factor genes, providing a hypothetical mechanism by which estradiol can alter coagulation factor gene expression [36–39]. The Protein S gene has been demonstrated to have a near consensus ERE through which an effect on Protein S expression could occur but is unknown why estradiol would downregulate some coagulation factors e.g. Protein S and upregulate others [48]. However, through evolutionary mechanisms, such regulation has resulted in a prothrombotic state possibly to reduce the risk of bleeding during the ante-, peri- and post partum periods and in turn reduce maternal morbidity and mortality from bleeding. Overall, given the EREs present upstream of many coagulation factors and the known changes in coagulation on exposure to exogenous estrogens, this work has provided further evidence for the role that estrogens play in the development of the prothrombotic state.

There also appears to be a potential role for progestogens in the development of the prothrombotic state. Firstly, progestogens have

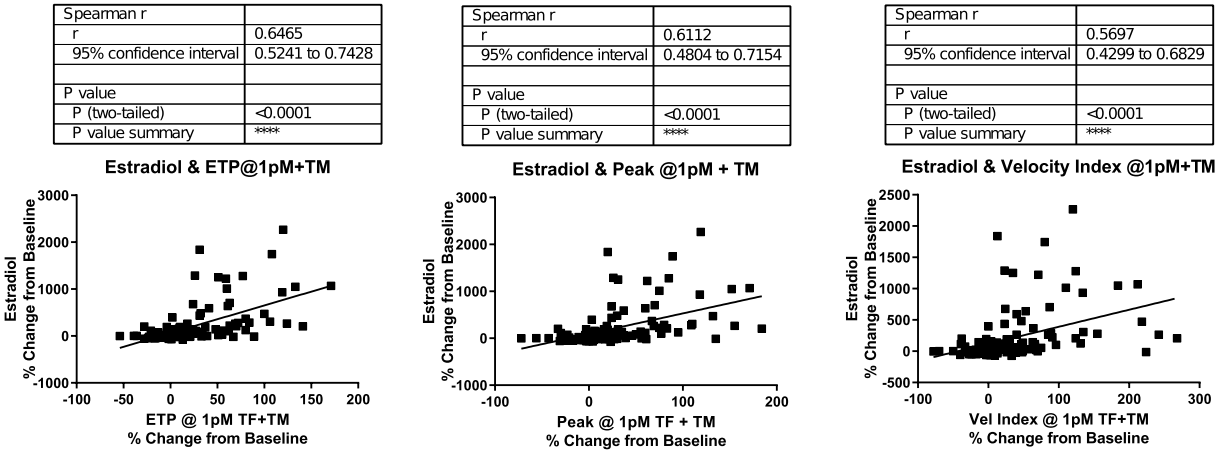

Fig. 4. Correlation of Estradiol with thrombin generation parameters ETP, Peak and Velocity Index under 1 pM TF + TM.
Percentage change from baseline is the change between baseline and a given time point in early pregnancy. TF: tissue factor; TM: thrombomodulin; ETP: endogenous thrombin potential.

Table 3
Correlation between parameters of thrombin generation, other markers of coagulation and the hormones, estradiol and progesterone.

	Estradiol		Progesterone		Protein S		Fibrinogen		D-dimer		Factor VIII	
	r	p value	r	p value	r	p value	r	p value	r	p value	r	p value
ETP	0.6465	< 0.0001	0.4979	< 0.0001	−0.6060	< 0.0001	0.5029	< 0.0001	0.3270	0.0090	0.5138	0.0001
Peak	0.6112	< 0.0001	0.4874	< 0.0001	−0.5233	< 0.0001	0.4682	0.0001	0.3460	0.0055	0.4656	0.0001
Vel Index	0.5697	< 0.0001	0.4104	0.0011	−0.4440	0.0003	0.4415	0.0003	0.3496	0.0050	0.4517	0.0002

been shown to affect coagulation factor expression in cell culture resulting in a prothrombotic phenotype and clinically it is known that certain exogenous progestogens alter thrombotic risk, with type and dose being important modifiers of risk [41–44,49]. Progestogens do not appear to have a direct effect on ERE expression but appear instead to have a modifying effect [50,51]. However, a direct effect is seen at high doses and this may be the reason why high dose norethistrone is directly prothrombotic in clinical trials. Interestingly progestogen receptors are membrane rather than nuclear and it may be that there are signalling pathways enabling crosstalk between the effects of the hormones on coagulation factor expression. It does have to be appreciated however that this is a complicated process of regulation and remains poorly understood. Finally, some effects of progestogens appear to be prothrombotic and some antithrombotic. This is why measuring thrombin generation is so important to be able to establish a phenotypic overview of the thrombotic state.

5. Limitations

We had very small numbers of individuals who could be assessed and it is possible with greater numbers that significant changes in coagulation factor and hormone levels could have been seen at an even earlier gestation than demonstrated in our work. This is particularly true of the hormone measurements where many women did not have sufficient plasma available to undertake these analyses. Sufficient plasma was available from most women for most of the coagulation factor assays. However, the hormonal assays were the last analyses to be undertaken at which point, for a number of women, there was insufficient plasma remaining. At Study Day +7 only 4 women had sufficient plasma to assess estradiol levels. With such small numbers a very large change in estradiol levels between the pre pregnant and pregnant state would have been required to demonstrate a significant increase. This is demonstrated by the fact that although estradiol levels had increased from pre pregnancy levels at Study Day +7 by 67% with very narrow confidence intervals, this was not a statistically significant change.

These women were not undergoing an entirely natural conception and it is possible this could have had an impact on the results. Firstly, these women had poor fertility although women were only selected if the pregnancy being studied resulted in an entirely normal outcome for both mother and baby. Secondly, embryos were being transferred and therefore the process of in vivo fertilization and conception did not occur and it cannot be entirely ruled out that certain mechanisms occur during spontaneous fertilization and conception which affect coagulation and hormonal levels. However, during this early stage of pregnancy, no exogenous hormones were administered to these women and the corpus luteum is responsible for hormonal production, both progesterone and estradiol. There is no reason to assume that the function of the corpus luteum in these women was different to that which would occur with a 'natural' conception.

6. Conclusion

Overall, we are the first to establish that the onset of the prothrombotic state occurs at a very early stage of pregnancy, within the first few weeks of gestation. We have also provided further evidence that the development of the prothrombotic state is likely due to the combined effect of estradiol and progestogens on coagulation factor production. This work would suggest that the increased risk of thrombosis beings very early in pregnancy. For this reason, consideration should be given to commencing thromboprophylaxis as early as possible in pregnancy, in women deemed at high risk of venous thrombosis.

By characterising coagulation and hormonal profiles in normal women, this enables the development of further work to try and establish whether the coagulation cascade influences poor pregnancy outcomes. Assessing individual components of the coagulation cascade has to date been ineffective at predicting poor pregnancy outcome. However, with the characterisation of thrombin generation, a global assay of coagulation, in early pregnancy, its role as a potential predictor of subsequent poor pregnancy outcome e.g. recurrent pregnancy loss, pre-eclampsia could now be investigated, with subsequent manipulation of abnormal thrombin generation patterns to determine whether poor outcomes can be reduced or averted.

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Conflict of interest

None for any author.

Addendum

C. N. Bagot designed and supervised the project. E. Leishman performed the experiments. C. N. Bagot and E. Leishman were involved in analyzing and interpreting the data and writing the manuscript. C. C. Onyiaodike collected the samples and critically reviewed the manuscript. V. B. Gibson performed the statistical analyses and critically

Table 4
Correlation between laboratory markers of coagulation and the hormones, estradiol and progesterone.

	Estradiol		Progesterone		Protein S		Fibrinogen		D-dimer	
	r	p value	r	p value	r	p value	r	p value	r	p value
Progesterone	0.4692	0.0002								
Protein S	−0.5833	< 0.0001	−0.6521	< 0.0001						
Fibrinogen	0.6200	< 0.0001	0.8306	< 0.0001	−0.6456	< 0.0001				
D-Dimer	0.4908	< 0.0001	0.5561	0.0006	−0.3977	0.0011	0.6121	< 0.0001		
Factor VIII	0.3173	0.0120	0.3334	0.0579	−0.2782	0.0260	0.3419	0.0057	0.2906	0.0198

reviewed the manuscript. F. Jordan and D. J. Freeman provided the samples and participant data and critically reviewed the manuscript.

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