Endogenous thrombin potential is higher during the luteal phase than during the follicular phase of a normal menstrual cycle

R. Chaireti¹,²,*, K.M. Gustafsson², B. Byström³, K. Bremme³,4, and T.L. Lindahl²

¹Department of Acute Internal Medicine and Coagulation Division, Linköping University Hospital, Linköping 581 85, Sweden ²Department of Clinical and Experimental Medicine, Linköping University, Linköping 581 85, Sweden ³Department of Women’s and Children’s Health, Division of Obstetrics and Gynecology, Karolinska Institutet, Stockholm 171 76, Sweden ⁴Department of Obstetrics and Gynecology, Karolinska University Hospital, Stockholm 171 76, Sweden

*Correspondence address. Tel: +46 103 0000; E-mail: roza.chaireti@lio.se

Submitted on December 31, 2012; resubmitted on February 24, 2013; accepted on March 7, 2013

STUDY QUESTION: Do thrombin generation and haemostatic parameters differ during the two phases of the menstrual cycle?

SUMMARY ANSWER: Total thrombin concentration is higher during the luteal phase compared with the follicular phase of the menstrual cycle.

WHAT IS KNOWN ALREADY: The coagulation cascade is affected by many variables, such as fluctuations in the levels of sex hormones. The studies on the variations in haemostatic parameters during the menstrual cycle have produced diverse results.

STUDY DESIGN, SIZE, DURATION: Thrombin generation and selected haemostatic parameters (fibrinogen, factor II, factor VII, factor VIII, factor X, von Willebrand factor, antithrombin and D-dimer) were measured during the two phases of a normal menstrual cycle in 102 healthy women not taking any form of hormone medication.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The study cohort consisted of 102 healthy women with regular menstrual cycles. Thrombin generation was measured by the calibrated automated thrombogram method. Progesterone and sex hormone-binding globulin were measured by chemiluminescence enzyme immunoassays. Estradiol was measured by a sensitive radioimmunoassay. Fibrinogen was measured by a clotting method, antithrombin was measured by a chromogenic method and factor II, factor VII, factor VIII, factor X, von Willebrand factor and D-dimer were measured by photometric methods.

MAIN RESULTS AND THE ROLE OF CHANCE: It was shown that the total amount of generated thrombin (Endogenous Thrombin Potential) was significantly higher during the luteal compared with the follicular phase (P = 0.027). Factor X was significantly higher during the follicular phase (P = 0.028). Progesterone exhibited significant associations (measured by the least squares regression analysis) with fibrinogen and factor X during the follicular phase (P = 0.043 and P = 0.033, respectively) and with factors II and VII during the luteal phase (P = 0.034 and P = 0.024, respectively). The validity of the results from the regression analysis was further confirmed by performing correlation analyses (Pearson correlation matrix) for haemostatic markers for the luteal and follicular phases (accepted correlation level >0.8).

LIMITATIONS, REASONS FOR CAUTION: The wide confidence interval for the differences in endogenous thrombin potential during the two phases could imply that the size of the cohort may not be sufficient to fully evaluate the biological variations. Additionally, the haemostatic markers were not shown to have significant associations with thrombin generation, suggesting that the increased thrombin concentration during the luteal phase would be mediated by another mechanism, as yet unidentified.

WIDER IMPLICATIONS OF THE FINDINGS: The associations between progesterone and the haemostatic markers, as shown for both phases of the menstrual cycle, suggest a previously unknown or undefined yet potentially significant role for progesterone in the coagulation system. However, it has been shown that the use of progestogen-only preparations does not affect the coagulation system, which is partly the reason why they are considered safe for women with thrombophilia or previous thrombotic event. Further studies are required in order to demonstrate whether our results can be extrapolated for synthetic progestins, which might have significant implication on the indications for their use.
Introduction

It is well known that the coagulation cascade is influenced by the physiological variations in the levels of female sex steroids and this is particularly evident during pregnancy (Astedt et al., 1998; Suzuki and Morishita, 1998) and less profound, but present nonetheless, during the menstrual cycle (Kadir et al., 1999). This is important, as it is known that the use of oral contraceptives (OCs) and hormone replacement therapy as well as pregnancy cause acquired activated protein C (APC) resistance (de Visser et al., 1999; Rosing et al., 1999). APC resistance, acquired or inherited as in carriers of factor V Leiden (FV Leiden), increases the risk for venous thromboembolism (VTE) (de Visser et al., 1999; Rosing et al., 1999). In this study, we aim towards a structured characterization of the haemostatic changes and the dynamics of thrombin generation during the two phases of the menstrual cycle.

The length of the menstrual cycle is an average of 28 days and consists of three phases: the follicular phase [cycle day (cd) 1–13], ovulation (~cd 14) and the luteal phase (cd 15–28). Estradiol concentrations are lowest on cd 1–3 and highest on cd 13–15 (late follicular phase and ovulation), followed by a decrease during the luteal phase. Progesterone concentrations are lowest on cd 1–8 (follicular phase) and highest on cd 21–25 (luteal phase) (Blombäck et al., 1997; Mihm et al., 2011). The sex hormone-binding globulin (SHBG) is a glycoprotein that binds to testosterone and estradiol and regulates their bioavailability; it is not subject to cyclic variations during the menstrual cycle (Rosner et al., 1999).

A number of studies have attempted to evaluate the changes in the haemostatic potential during the menstrual cycle. The design and the results of those studies are diverse. It has been repeatedly (Jern et al., 1991; Kadir et al., 1999; Miller et al., 2002; Roell et al., 2007) reported that von Willebrand factor levels are lowest during menstruation or in the early follicular phase. There are, however, other studies which have found no variations (Onundarson et al., 2001; Giardina et al., 2004; Koh et al., 2005). It has also been suggested that FVIII reaches its peak level around the time of the ovulation (Mandalaki et al., 1980), but most studies report no cyclic variation (Onundarson et al., 2001; Koh et al., 2005; Knol et al., 2012). Giardina et al. reported that the fibrinolytic end-product, D-dimer was significantly higher during the follicular phase (Giardina et al., 2004). On the contrary, Koh et al. (2005) showed that the lowest levels of D-dimer were present in the late follicular phase. Another haemostatic parameter that has been thoroughly studied is fibrinogen. A recent review by Knol et al. (2012) included 20 studies on the cyclic variations of fibrinogen. Of those studies, 12 reported no variations, whereas 6 showed that the fibrinogen levels were lowest during the follicular or middle-cycle phase and the remaining two studies showed that the levels were lowest during the luteal phase. Antithrombin (AT) has been shown to be rather constant during the menstrual cycle (Lebech and Kjaer, 1989; Siegbahn et al., 1989; Lebech et al., 1990).

The associations between the levels of the female sex hormones and the haemostatic potential have been studied mainly in the context of treatment with various contraceptive medications, where the hormone effect on haemostasis has been shown to depend mostly on estrogen content (Sabra and Bonnar, 1983; Daly and Bonnar, 1990). Initiation of estrogen therapy increases prothrombin fragment 1+2 (F1 +2) and fibrinopeptide A (FPA) and decreases free protein S in healthy post-menopausal women (Scarabin et al., 1997; Caine et al., 1992; Luyer et al., 2001), whereas the use of OC has a positive effect on the levels of procoagulant proteins and a negative effect on the levels of coagulation inhibitors (Kluft and Lansink, 1997; Winkler, 1998). The effects of sex steroids on coagulation are more dramatic when great amounts of hormones are introduced rapidly, as in the use of the ‘day-after’ pill, with the procoagulant effects being even more pronounced with preparations containing estrogens (van Rooijen et al., 2007a,b). Andersson et al. (1997) showed that the concentrations of factor VII (FVII), factor X (FX), fibrinogen and plasminogen at a low estradiol or progesterone level can predict the corresponding concentrations of those coagulation proteins at a high hormone level.

The effects of physiological concentrations of sex steroids on haemostasis are not as thoroughly studied as the effects of hormone medications. Blombäck et al. (1992) showed considerable variations in plasminogen and vWF antigen (vWF:Ag) in 15 women during the menstrual cycle but could not demonstrate any relation between these two haemostatic markers and estradiol, progesterone or testosterone.

The data on the variations of thrombin generation during the menstrual cycle are scarce. Mainly surrogate thrombin generation markers, such as F1 +2 and thrombin–AT complex (TAT), have been employed. Both TAT (Usznyski, 1997) and F1 +2 (Feuring et al., 2002; Blombäck et al., 2007) have been shown to remain rather constant during the menstrual cycle.

The issue of which factors affect thrombin generation the most is still controversial. Brummel-Ziedens et al. (2005) reported that only factor II (FII) affects the total amount of generated thrombin, whereas maximum (peak) thrombin concentration is affected by many factors in platelet poor plasma (PPP). Machlus et al. (2009) showed that in platelet-free plasma, factor XI (FXI), factor IX (FIX), FVIII, FX, FII and fibrinogen had a significant positive correlation with peak thrombin concentration and with the endogenous thrombin potential (ETP, total thrombin concentration).

Our study focuses on the variations of thrombin generation and selected haemostatic parameters during the two phases of a normal menstrual cycle in healthy women not using any form of hormone medications. Additionally, we investigate the effect of sex hormones and SHBG levels on haemostasis and thrombin generation, in an attempt to elucidate the clinical reality of hypercoagulability associated with, or mediated by, increased concentrations of those hormones, in particular estradiol.
**Materials and Methods**

**Study participants**

Our original study cohort consisted of 104 healthy women (mean age 31.7 ± 8.6 and mean BMI 23.1 ± 3) who volunteered for the study. The inclusion process has been described in detail previously (van Roojen et al., 2007a, b). Inclusion criterion was a history of regular menstrual cycles with a cycle length varying between 21 and 35 days. The exclusion criteria were pregnancy, hormonal therapy or breast-feeding within 2 months prior to the study start. There were no restrictions with respect to age, BMI, smoking or number of previous pregnancies. The menstrual cycle length (in days) was 28.2 ± 2.0 (mean ± SD) with a range of 25–34 days. The number of bleeding days was 4.8 ± 1.2 (mean ± SD).

None of the participants in the study had suffered a thromboembolic event, however eight women had at least one first degree relative with a history of VTE. A total of 86 participants had combined OC (COCs) at some point prior to the inclusion. Twelve subjects were heterozygous carriers of the FV Leiden mutation. None were homozygous carriers. Of the original cohort of 104 women, 2 were excluded: 1 due to severe problems with the blood sampling and 1 withdrew her consent due to personal reasons.

**Blood sampling and handling**

Blood samples were collected during the follicular phase (cd 3–5) and during the luteal phase (cd 22–25) of the menstrual cycle from each volunteer. Venous blood samples were drawn from an antecubital vein after 15 min in the supine position. All samples were drawn in the morning after an overnight fast. Blood samples for analysis of coagulation factors were collected in tubes containing 0.13 mM citrate (Vacutainer, Becton Dickinson, Meylen, France) and immediately centrifuged at 2000g for 15 min. After removal of the cells, plasma was re-centrifuged for another 15 min at 2000g. Blood for serum preparation was collected in plain Vacutainer tubes without anticoagulants and kept at room temperature for 1 h before centrifugation at 2000g for 10 min. Cell-free plasma and serum samples were stored at −70°C until analysed.

**Measurement of thrombin generation by the calibrated automated thrombogram**

Of the 102 subjects included in the study, we obtained full thrombin generation profiles (for both phases of the menstrual cycle) for 73. Each sample was measured in triplicate. About 20% of the missing thrombin generation profiles were the result of errors during measurement (‘flat thrombin generation curve’). The remaining 80% of the missing profiles was due to either limited available plasma volume (not enough in order to perform the measurement in triplicate), or to the absence of a valid thrombin generation measurement for one of the two phases of the menstrual cycle for one subject. This means that if we were not able to obtain thrombin generation profiles for both phases of the menstrual cycle for one participant, this participant was excluded from the thrombin generation analysis.

The first author performed all thrombin generation measurements. The samples used were PPP obtained from whole blood as described above. The frozen plasma was thawed by immersion into a water bath at 37°C immediately prior to analysis and the same procedure was followed for all the samples. Thrombin generation was measured by the calibrated automated thrombogram method as described in the Thrombogram Guide by Thrombinoscope BV (Maastricht, the Netherlands). The mixture of PPP reagent (trigger) and PPP used in the assay contained 5 pM tissue factor (TF) and 4 μM phospholipids. The markers calculated were lagtime (clotting time, the moment at which thrombin generation begins), ETP the total amount of generated thrombin, in nanomolar*minute), peak height (maximal thrombin concentration, in nanomolar thrombin) and tPeak (time to peak height, i.e. reach maximal thrombin concentration, in minutes). All reagents were obtained from Thrombinoscope BV, Maastricht, The Netherlands. The 96-well plates used were obtained from Ninolab, Stockholm, Sweden.

**Measurement of hormones and sex hormone-binding globulin**

Serum concentrations of progesterone and SHBG were determined by the direct chemiluminescence enzyme immunoassays Immulite 1000 using commercial kits from Diagnostic Products Corporation, Los Angeles, CA. Estradiol levels were analysed by a sensitive Spectria radioimmunoassay from Orion Diagnostica (Espoo, Finland). The detection limits and within- and between-assay coefficients of variations for progesterone were 0.6 nmol/l, 9.6 and 9.9%; for SHBG 0.2 nmol/l, 6.5 and 8.7% and for estradiol 5 pmol/l, 7.4 and 10%, respectively.

**Measurement of haemostatic parameters**

Fibrinogen was measured by a clotting method using commercial kits and reagents from Instrumentation Laboratory (IL). Milan, Italy. AT was measured by a chromogenic method using commercial kits and reagents from IL. D-dimer was measured by a photometric method using reagents from MediRox, Nyköping, Sweden and IL. Factors II, VII, VIII and X were measured by a photometric method using commercial kits and reagents from IL. All of the aforementioned measurements were performed by the ACL TOP instrument, IL. Von Willebrand factor was measured by the Technozym® vWF:Ag ELISA from Technoclone GmbH, Vienna, Austria.

**Statistical analysis**

All the results were analysed by SPSS, Systat, Inc. Software. When the number of observations (n) was >30, we used the paired t-test (P < 0.05, CI 95%) in order to determine the differences in the levels of thrombin generation markers and coagulation parameters in the luteal and follicular phase for each volunteer. When the n was lower than 30, we used Mann–Whitney U-test (P < 0.05, CI 95%). The correlations between hormones and thrombin generation markers/haemostatic markers were evaluated by simple correlation method for continuous data (Pearson’s).

We used the least squares multiple regression analysis to determine the associations between estradiol, progesterone, SHBG, thrombin generation markers and haemostatic variables, as well as the influence of haemostatic variables on thrombin generation markers. The validity of the results from the regression analysis was further confirmed by performing correlation analyses (Pearson correlation matrix) for thrombin generation markers and coagulation factors for the luteal and follicular phase (accepted correlation level >0.8).
We used multiple regression analysis in order to evaluate the associations between the haemostatic parameters and thrombin generation markers. During the follicular phase, AT and von Willebrand factor had the greatest association with lagtime (P = 0.018 and P = 0.053, respectively, least square multiple regression analysis). Von Willebrand factor exhibited a significant interaction with peak thrombin concentration (P = 0.024, least square multiple regression analysis). ETP interacted mainly with FII and AT (P = 0.131, respectively P = 0.149, least square multiple regression analysis).

During the luteal phase, lagtime was associated, albeit not significantly, with FVIII (P = 0.132) and AT (P = 0.149, least square multiple regression analysis). The interactions of the remaining haemostatic variables with lagtime as well as the other thrombin generation parameters did not reach statistical significance. No strong correlations between the thrombin generation markers and the haemostatic parameters were found.

**Results**

**Thrombin generation during the follicular and luteal phases of the menstrual cycle**

We were able to obtain complete TG profiles (TG data from both phases of the menstrual cycle) for 73 women. The levels of the thrombin generation markers and the haemostatic parameters measured are presented in Table I. ETP was slightly but significantly higher during the luteal phase compared with the follicular phase, 1609 versus 1524 nanomolar* min (P = 0.027, paired t-test). The other markers did not exhibit any significant differences. No statistically significant differences were found for the thrombin generation markers during the follicular and luteal phases for the carriers and non-carriers of the FV Leiden (Mann–Whitney test).

**Haemostatic parameters during the follicular and luteal phases of the menstrual cycle**

FX (P = 0.028, paired t-test) was higher during the follicular phase compared with the luteal phase. The levels of FVII and D-dimer were also slightly, although not significantly higher during the follicular phase (P = 0.09, respectively, P = 0.067, paired t-test).

**Associations between the haemostatic parameters and thrombin generation markers**

We used multiple regression analysis in order to evaluate the associations between the haemostatic parameters and the thrombin generation markers. During the follicular phase, AT and von Willebrand factor had the greatest association with lagtime (P = 0.018 and P = 0.053, respectively, least square multiple regression analysis). Von Willebrand factor exhibited a significant interaction with peak thrombin concentration (P = 0.024, least square multiple regression analysis). ETP interacted mainly with FII and AT (P = 0.131, respectively P = 0.149, least square multiple regression analysis).

During the luteal phase, lagtime was associated, albeit not significantly, with FVIII (P = 0.132) and AT (P = 0.149, least square multiple regression analysis). The interactions of the remaining haemostatic variables with lagtime as well as the other thrombin generation parameters did not reach statistical significance. No strong correlations between the thrombin generation markers and the haemostatic parameters were found.

**Table I Haemostatic variables, thrombin generation markers, sex hormones and SHBG in the luteal and follicular phase of the menstrual cycle (expressed as median ± SD)**

<table>
<thead>
<tr>
<th></th>
<th>Follicular phase</th>
<th>Luteal phase</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lag-time (min)</td>
<td>2.59 ± 0.4</td>
<td>2.67 ± 0.5</td>
<td>0.261</td>
</tr>
<tr>
<td>ETP (nM*min)</td>
<td>1524.5 ± 283.2</td>
<td>1609.2 ± 342.8</td>
<td>0.027</td>
</tr>
<tr>
<td>Peak (nM)</td>
<td>300.1 ± 50</td>
<td>308.1 ± 54.4</td>
<td>0.195</td>
</tr>
<tr>
<td>Tpeak (min)</td>
<td>5.2 ± 0.7</td>
<td>5.3 ± 0.7</td>
<td>0.715</td>
</tr>
<tr>
<td>PGN (nmol/l)</td>
<td>2.9 ± 4.3</td>
<td>31.9 ± 22</td>
<td></td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>56.3 ± 18.3</td>
<td>58.1 ± 22.8</td>
<td></td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td>116.7 ± 85</td>
<td>346.6 ± 203.4</td>
<td></td>
</tr>
<tr>
<td>FL (kIU/l)</td>
<td>1 ± 0.1</td>
<td>1 ± 0.1</td>
<td>0.318</td>
</tr>
<tr>
<td>AT (kIU/l)</td>
<td>1 ± 0.07</td>
<td>1 ± 0.08</td>
<td>0.337</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>2.6 ± 0.4</td>
<td>2.9 ± 1.7</td>
<td>0.215</td>
</tr>
<tr>
<td>D-dimer (mg/l)</td>
<td>0.1 ± 0.1</td>
<td>0.07 ± 0.06</td>
<td>0.067</td>
</tr>
<tr>
<td>FVII (kIU/l)</td>
<td>0.9 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>0.09</td>
</tr>
<tr>
<td>FX (kIU/l)</td>
<td>1 ± 0.7</td>
<td>1 ± 0.3</td>
<td>0.828</td>
</tr>
<tr>
<td>VWF (kIU/l)</td>
<td>0.9 ± 0.4</td>
<td>0.9 ± 0.3</td>
<td>0.256</td>
</tr>
</tbody>
</table>

**Thrombin generation, haemostatic parameters and their association with the female sex hormones and the sex hormone-binding globulin during the menstrual cycle**

Overall, the correlations between thrombin generation markers, haemostatic parameters, sex hormones and SHBG were weak for both phases. The least square multiple regression analyses showed that progesterone had a significant association with fibrinogen (P = 0.043), FX (P = 0.033) and a marginally significant interaction with FVIII (P = 0.056, least square multiple regression analysis) during the follicular phase. Progesterone exhibited similarly significant associations with FII and FVII (P = 0.034 and P = 0.024, respectively, least square multiple regression analysis) and was weakly related to D-dimer (P = 0.07, least square multiple regression analysis) during the luteal phase. Neither sex steroids nor SHBG had any significant interactions with the thrombin generation markers during the follicular or luteal phase.

**Discussion**

Our results show that thrombin generation is significantly higher during the luteal phase of the menstrual cycle compared with the follicular phase (Table I). Thrombin generation markers are associated with different haemostatic parameters during the two phases of the menstrual cycle depending on the specific thrombin generation parameter that is studied. These associations did not always reach statistical significance, but they nonetheless express physiological variations in thrombin generation profiles rather than isolated markers.

The haemostatic variables measured in our study were chosen as they have been previously shown to affect thrombin generation (Brummel-Ziedins et al., 2005; Machlus et al., 2009) and/or have been studied in relation to the menstrual cycle (Knol et al., 2012). Our results show a discreet tendency towards higher haemostatic activity during the follicular phase, in terms of procoagulant markers (higher FX and FVII, P = 0.028 and P = 0.09, respectively) and fibrinolytic activity (higher D-dimer, P = 0.067); however, these differences did not always reach statistical significance. Menstrual bleeding is associated with an increase in local fibrinolysis due to elevated levels of endometrium-derived plasmin and plasminogen activators (Gleeson, 1994). The samples were drawn early during the follicular phase.
significant effect on the results. Moreover, we hypothesize that residual systemic increased fibrinolysis during the late menstruation could be a plausible explanation behind the higher D-dimer. Although the increases in FVII and FX are not similarly easy to explain, it has been reported previously that the activated FVII (FVIIa) is higher during the follicular phase (Kapiotis et al., 1998). AT was not statistically different during the two phases; however, we did not study the effect of other important anticoagulant proteins such as protein C.

During the luteal phase of the menstrual cycle, it was progesterone that had the greatest influence on haemostasis, which is consistent with the fact that the increase in the levels of progesterone during this phase is relatively greater than the simultaneous increase in estradiol. The multiple regression analysis showed that progesterone had significant associations with fibrinogen, FVIII (marginally) and FX during the follicular phase and with FII and FVII during the luteal phase. Although FII, FVII, FVIII, FX and fibrinogen have been reported to play central roles for thrombin generation (Mann et al., 2003; Brummel-Ziedins et al., 2005; Machlus et al., 2009), this was not confirmed in our study. It has been suggested that progesterone could affect platelet function during the menstrual cycle (Roell et al., 2007); however, its influence on coagulation is not that well defined. Blombäck et al. (1997) showed that progesterone did not have any significant effect on haemostasis. Our results demonstrate that progesterone potentially has a more important role than previously believed. Uncovering the effects of progesterone on coagulation could have significant clinical implications. Previous studies have demonstrated no procoagulant haemostatic changes resulting from the use of progestogen-only preparations (Kemmeren et al., 2001, 2004), which is partly the reason why they considered safe for women with thrombophilia or previous thrombotic episodes in contrast to estrogen-containing preparations (Sabra et al., 2001, 2004), which is partly the reason why they considered safe for women with thrombophilia or previous thrombotic episodes in contrast to estrogen-containing preparations (Sabra and Bonnar, 1983). However, at this point it is uncertain whether our results on the effect of progesterone can be extrapolated even for synthetic progestins.

In this study we performed a structured analysis of the relations between haemostatic markers, thrombin generation, female sex steroids and SHBG, thus providing a rather adequate surrogate system for the physiological changes taking place during the normal menstrual cycle. The majority of the related studies concentrate solely on the differences in coagulation parameters during the menstrual cycle and some authors even measure hormone levels, but, to our knowledge, this is the first time that thrombin generation measured in real time (and not expressed via surrogate markers), haemostatic variables, female hormones and SHBG are studied simultaneously and in relation to each other.

One of the strengths of our study is that our cohort is greater than the majority of the cohorts used in other studies with similar design (Knol et al., 2012). In a recent review, Knol et al. (2012) report that most of the studies in the field have used relatively small cohorts with a mean size of around 40–50.

Our results were not affected by the use of hormone medications. However, we did include subjects who were heterozygous for the FV Leiden mutation, but their small number is as expected for our Swedish population (Lindahl et al., 1999) and should not have any significant effect on the results. One weakness of our study is that our results did not always reach statistical significance; a type II error cannot be excluded. Additionally, the wide confidence interval for the differences in ETP during the two phases (data not shown) could imply that the size of the cohort may not be sufficient to fully evaluate the biological variations. The haemostatic markers that were significantly associated with progesterone were not shown to have a similar interaction with thrombin generation.

During the luteal phase thrombin generation was increased, but the coagulation cascade was marginally faster during the follicular phase. This implies that the observed increase in ETP could be mediated by another mechanism.

The coagulation system is extremely complicated and affected by many diverse factors that in turn interact with one another. For example, Jern et al. (1991) showed that the combination of mental stress and female hormones further exacerbates the differences in some haemostatic parameters (fibrinogen, von Willebrand factor, tissue plasminogen activator), with those changes being more pronounced in the luteal phase. Coagulation factors also exhibit circadian variations (Haus, 2007) and it is very difficult to co-evaluate those fluctuations. As coagulation activation is profoundly affected by inflammation (Chen and Dorlin, 2009; Levi, 2010), studying inflammation markers in relation to thrombin generation during the menstrual cycle could help to complete the picture. At the same time, the significant effect of progesterone on coagulation that was shown in our study need to be further investigated in larger cohorts.

Authors’ roles
R.C. initiated the study, took part in planning and writing of the manuscript, wrote the first draft of the manuscript and performed the thrombin generation analysis. K.M.G. contributed to revising the article and to analysis and interpretation of data (haemostatic markers). B.B. contributed to revising the article and to analysis and interpretation of data (hormones). K.B. was responsible for recruiting the study participants, participated in planning of the study and in the writing of the manuscript. T.L.L. participated in planning of the study and in writing of the manuscript and directing the analysis of coagulation factors.

Funding
This study was supported by the Karolinska Institutet, Linköping University and the County Council of Östergötland.

Conflict of interest
None declared.

References
Thrombin generation during the menstrual cycle

1851


Usynski N. Generation of thrombin in blood plasma of non-pregnant and pregnant women studied through concentration of