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### Low thrombin generation in users of a contraceptive containing estetrol and drospirenone

Morimont, Laure; Jost, Maud; Gaspard, Ulysse; Foidart, Jean Michel; Dogné, Jean Michel; Douxfils, Jonathan

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1 **LOW THROMBIN GENERATION IN USERS OF A CONTRACEPTIVE CONTAINING ESTETROL AND DROSPIRENONE**

2  
3 **Short title:** Thrombin generation and estetrol

4  
5 Laure Morimont<sup>1,2</sup>, Maud Jost<sup>3</sup>, Ulysse Gaspard<sup>4</sup>, Jean-Michel Foidart<sup>3,5</sup>, Jean-Michel Dogné<sup>2</sup>, Jonathan Douxfils<sup>1,2</sup>

6  
7 <sup>1</sup>Qualiblood sa, Namur, Belgium; <sup>2</sup>University of Namur, Faculty of Medicine, Department of Pharmacy, Namur  
8 Research Institute for Life Sciences (NARILIS), Clinical Pharmacology Research Group, Namur, Belgium; <sup>3</sup>Estetra SRL,  
9 An affiliate's Company of Mithra Pharmaceuticals, Liège, Belgium, <sup>4</sup>Department of Obstetrics and Gynecology,  
10 University of Liège, Belgium; <sup>5</sup> University of Liège, Liège, Belgium.

11  
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14 **Corresponding author:**

15 Laure Morimont, PharmD

16 <sup>1</sup>Qualiblood sa, Namur, Belgium;

17 <sup>2</sup>University of Namur, Faculty of Medicine, Department of Pharmacy, Namur Research Institute for Life Sciences  
18 (NARILIS), Namur Thrombosis and Hemostasis Center (NTHC), Namur, Belgium

19 Rue du Séminaire 20a, 5000 Namur, Belgium

20 Mail: laure.morimont@qualiblood.eu

21 Phone: +32 81 44 49 92

22 ORCID: 0000-0001-9195-304X

23  
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1 **ABSTRACT**

2  
3 **Objective:** To compare the impact on thrombin generation of the new combined oral contraceptive containing 15  
4 mg estetrol and 3 mg drospirenone with ethinylestradiol (30 or 20 mcg) associated either with 150 mcg  
5 levonorgestrel or with 3 mg drospirenone.

6 **Methods:** Data were collected from the "E4/DRSP Endocrine Function, Metabolic Control and Hemostasis Study"  
7 (NCT02957630). Overall, the per protocol set population included 24 subjects in the ethinylestradiol/levonorgestrel  
8 arm, 28 subjects in the ethinylestradiol/drospirenone and 34 subjects in the estetrol/drospirenone arm.  
9 Thrombograms and thrombin generation parameters (lag time; peak; time to peak; endogenous thrombin potential  
10 [ETP] and mean velocity rate index [mVRI]) were extracted for each subject at baseline and after 6 cycles of  
11 treatment.

12 **Results:** After 6 cycles of treatment, ethinylestradiol-containing products arms show a mean thrombogram outside  
13 the upper limit of the reference range, that is the 97.5<sup>th</sup> percentile of all baseline thrombograms. On the other  
14 hand, the mean thrombogram of estetrol/drospirenone is within this reference interval. After 6 cycles of  
15 treatment, all thrombin generation parameters are statistically less impacted by estetrol/drospirenone than  
16 ethinylestradiol-containing products.

17 **Conclusions:** In conclusion, an association of 15 mg estetrol with 3 mg drospirenone does not have an impact on  
18 thrombin generation compared to ethinylestradiol-containing products which, either associated with  
19 levonorgestrel or drospirenone, are able to increase the production of procoagulant factors and decrease the  
20 production of anticoagulant ones, shifting the patient to a prothrombotic state. Ethinylestradiol-containing  
21 products thus generate prothrombotic environments contrary to estetrol which demonstrates a neutral profile on  
22 hemostasis.

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24

## 1 MAIN MANUSCRIPT

### 2 INTRODUCTION

3 Pregnancy and postpartum, as well as exogenous hormones exposure, such as combined hormonal contraceptives  
4 (CHCs), create hormonal changes associated with an increased risk of venous thromboembolism (VTE) (1). Indeed, a  
5 5-fold increased risk of VTE is reported during pregnancy, and up to a 20- to 60-fold increased risk in the  
6 postpartum period (i.e., during the first 6 weeks after delivery) (2-4). For women using CHCs, the relative risk varies  
7 between 1.3 to 5.6 depending on the estroprogestative association and the dose of the estrogenic component (5-  
8 11).

9 Pregnancy and the use of CHCs cause changes in plasma levels of almost all proteins involved in the coagulation  
10 and fibrinolysis (12). These changes might be considered as relatively modest when measured separately but they  
11 could have a supra-additive effect leading to a procoagulable state responsible for this increased risk of VTE (13).  
12 Overall, rises in coagulation factors II, V, VII, VIII, IX, X, XI, XII and von Willebrand factor, as well as fibrinogen levels  
13 are observed (12). On the other hand, antithrombin, protein S and tissue factor pathway inhibitor (TFPI) levels,  
14 three proteins contributing to the anticoagulant system, are decreased (14-17). As for the fibrinolysis, there is an  
15 increase in plasminogen levels but a decrease in tissue plasminogen activator antigens and plasminogen activator  
16 inhibitor-1 levels (12). These hormonal changes, both during pregnancy and following the use of hormonal therapy,  
17 are also associated with activated protein C (APC) resistance which can result from increases in FII, FVIII or FX levels  
18 and/or decreases in protein S and TFPI (12,13,18,19).

19 Among assays measuring APC resistance, the endogenous thrombin potential (ETP)-based APC resistance assay is  
20 the most sensitive towards acquired APC resistance and has been linked to an increased risk of VTE in women on  
21 hormonal therapy (19-21). This technique relies on the thrombin generation assay (TGA) which permits to obtain a  
22 thrombogram, i.e., a visual and quantitative representation of the amount of thrombin generated over time in a  
23 cupule. While the normalized APC sensitivity ratio (nAPCs<sub>r</sub>) reflects the capacity of the ETP-parameter  
24 (representing the area under the thrombogram) to be reduced in presence of exogenous APC, other parameters of  
25 the thrombogram can be exploited (22). Indeed, they can provide information on the prothrombotic tendency,

1 (23,24) independently of the resistance towards exogenous APC. Besides, the use of ethinylestradiol (EE) based-  
2 CHCs, and other known hypercoagulable states, have been shown to enhance in vitro thrombin generation (25-27).  
3 A combination of 15 mg estetrol (E4) and 3 mg drospirenone (DRSP) (Nextstellis® in the US, Drovelis® and Lydisilka®  
4 in Europe) has recently been approved (28). Estetrol is a natural and native fetal estrogen synthesized exclusively in  
5 the human fetal liver (29). It has a unique mode of action, different from those of other estrogens, by activating the  
6 nuclear estrogen receptor  $\alpha$  (Er $\alpha$ ) but antagonizing the membrane Er $\alpha$  (30). The use of E4 demonstrated a low  
7 impact on the liver with minimal effects on lipids, lipoproteins, sex hormone binding globulin (SHBG) and several  
8 coagulation and fibrinolytic proteins (31). The association of E4 with DRSP also showed a much lower impact on  
9 APC resistance compared to EE with levonorgestrel (LNG) or EE with DRSP as well as on the level of prothrombin  
10 fragment 1+2, a marker of the ongoing coagulation (31). Nevertheless, while some coagulation factors like  
11 prothrombin, FVII, TFPI or protein S were individually impacted by each of these therapies (i.e., E4/DRSP, EE/LNG  
12 and EE/DRSP), the synergistic effect of these changes on the hemostasis could not be captured by these singular  
13 measurements. Therefore, a global test capable of capturing all pro- and anticoagulants factors levels changes,  
14 would allow a more accurate evaluation of the impact of a CHC on hemostasis and the associated risk of VTE. The  
15 thrombin generation test permits to assess the coagulation process in its entirety and it has been shown to be  
16 sensitive to the synergistic hemostatic alterations induced by CHCs (32). This study aims therefore at comparing the  
17 impact of E4/DRSP with EE/LNG and EE/DRSP on thrombin generation.

## 18 MATERIAL AND METHODS

### 19 Study design

20 This single center, randomized, open-label, controlled, three-arm, parallel study in healthy females was conducted  
21 from September 2016 through October 2017 at Dinox BV, Groningen, the Netherlands (Eudra CT 2916-001316-37,  
22 Clinicaltrials.gov NCT02957630). The study, performed in accordance with the Declaration of Helsinki and the  
23 International Council for Harmonization (ICH) E6 (R2) Good Clinical Practice guidelines, was approved by an  
24 independent local ethics committee and written informed consent was obtained from all participants before study  
25 entry. The study consisted of a pretreatment cycle (baseline), followed by 6 28-day treatment cycles. A total of 100  
26 healthy women (40 in the investigational group and 30 per comparator group) was planned to be included in the

1 study. Visits were planned to be at screening, at randomization/baseline, at cycle 3, at cycle 6 and/or at the end of  
2 the study. As hemostatic results at cycle 3 were similar to those of cycle 6, only data obtained at cycle 6 will be  
3 reported.

#### 4 **Study population**

5 Healthy females aged 18-50 years with a body mass index between 18 and 30 kg/m<sup>2</sup>, and a natural menstrual cycle  
6 of maximum 35 days were eligible for inclusion. Main exclusion criteria were contraindications for the use of  
7 hormonal contraceptives, known coagulopathy or thrombogenic mutation, the use of anticoagulants or other drugs  
8 affecting coagulation and platelet aggregation and an abnormal Papanicolaou smear test. The use of an injectable  
9 contraceptive was not allowed within 3-10 months prior the screening, depending on the type of injection. Women  
10 with CHC use prior to the study had a washout period of 4 weeks before pretreatment cycle. Pretreatment cycle  
11 started on the first day of menstrual cycle (following the washout cycle for former CHC users). All subjects started  
12 the intake of the active study medication on the first day of their menstrual cycle following the pretreatment cycle.

#### 13 **Study treatment**

14 Eligible subjects were stratified by previous hormonal contraceptive use (8 weeks or >8 weeks without use before  
15 study treatment start) and by age (≤35 years or >35 years of age). Subjects were then assigned, using a  
16 computerized random allocation sequence, to one of the following treatments in a 4:3:3 ratio: 15 mg E4 (as  
17 monohydrate, equivalent to 14.2 mg anhydrate) combined with 3 mg DRSP (E4/DRSP; 24-day active/4-day placebo  
18 regimen); 30 mcg EE combined with 150 mcg LNG (EE/LNG; 21-day active/7-day placebo regimen), or 20 mcg EE  
19 combined with 3 mg DRSP (EE/DRSP; 24-day active/4-day placebo regimen). The E4/DRSP-containing product was  
20 manufactured by Haupt Pharma, Münster, Germany and provided by Estetra SRL, an affiliate's company of Mithra  
21 Pharmaceuticals, Liège, Belgium. The other two products, i.e., EE/LNG (Melleva® 150/30, Leon Farma) and EE/DRSP  
22 (Yaz®, Bayer Healthcare) were obtained from a local pharmacy. Study treatment started on the first day of  
23 menstrual cycle following the pretreatment cycle. Treatment compliance was verified using a diary and check of  
24 returned packages.

25

## 1                    **Study assessment and outcome parameters**

2 Hemostasis parameters (fibrinogen, prothrombin, factor VII, FVIII, von Willebrand factor, antithrombin, protein S  
3 activity, free protein S, protein C, free TFPI, plasminogen, plasminogen activator inhibitor type-1 (PAI-1), tissue  
4 plasminogen activator (tPA), ETP-based APC resistance (expressed as nAPCsr), D-dimers and prothrombin fragment  
5 1+2) as well as SHBG have previously been reported (31).

6 Thrombin generation assay has been performed on a Calibrated Automated Thrombogram (CAT) (Diagnostica  
7 Stago, Asnières-sur-Seine, France) using the STG-ThromboScreen® (Diagnostica Stago) as triggering reagent. A  
8 complete description of the method is reported in detail elsewhere (22). Thrombogram parameters were  
9 integrated using the Thrombinoscope software (Thrombinoscope bv, version 5.0) and the following TGA  
10 parameters were extracted from the thrombin generation curve: i) the lag-time, corresponding to the start of  
11 thrombin generation (expressed in min), ii) the peak height, corresponding to the maximal concentration of  
12 thrombin generated (expressed in nM), iii) the time-to-peak, corresponding to the time to reach the peak  
13 (expressed in min), iv) the endogenous thrombin potential (ETP), corresponding to the area under the curve  
14 (expressed in nM\*min) and v) the mean velocity rate index, corresponding to the maximal rate of thrombin  
15 generation (expressed in nM/min). **Figure 1** provides a representation of a thrombogram with the corresponding  
16 TGA parameters.

## 17                    **Statistical analysis**

18 Statistical analysis was performed using GraphPad version 9.3.1 (GraphPad Prism 9.3.1 for macOs, GraphPad  
19 Software, San Diego, California, USA, [www.graphpad.com](http://www.graphpad.com)). All randomized subjects who received at least one dose  
20 of the study medication and had at least one hemostasis assessment on treatment, without any major protocol  
21 deviation impacting the endpoints, were included in the analysis (per protocol dataset). Descriptive statistics were  
22 used to analyze the data (n, mean, standard deviation [SD], median, minimum-maximum range, 10-90<sup>th</sup> percentile,  
23 and 95% confidence intervals [95%CI]). Changes of the different TGA parameters from baseline to cycle 6 have  
24 been computed using paired t-test and differences between treatment groups for a particular timepoint were  
25 assessed using an ordinary one-way ANOVA followed by a Tukey's multiple comparison test. Reference ranges for  
26 thrombin generation and associated parameters are reported as the 2.5<sup>th</sup> – 97.5<sup>th</sup> percentile of the entire baseline

1 cohort (n=86), in accordance with the definition of the reference intervals as reported in the Clinical & Laboratory  
2 Standards Institute (CLSI) EP-28-A3C.(33). All statistical tests were evaluated with a level of significance of 0.05.

### 3 **RESULTS**

#### 4 **Study population**

5 A total of 143 subjects were screened for eligibility of which 101 were randomized, 98 received study treatment  
6 and 88 among these participants completed the study (per protocol set population) (**Figure 2**). A summary of the  
7 demographic data at study entry is presented in **Table 1** and shows no difference between groups at baseline.  
8 There was no important protocol deviation, including non-compliance issues. For one patient in the EE/DRSP group,  
9 there was no sufficient plasma sample and thrombin generation could not be performed. One patient in the  
10 EE/LNG group with a thrombin generation curve at baseline defined as outlier, was not included in TGA analyses.  
11 The final per protocol set population for this study was therefore 86 among which 24 subjects received EE/LNG, 28  
12 received EE/DRSP and 34 received E4/DRSP. Previous use of CHC concerned 38% of women in the EE/LNG group,  
13 43% in the EE/DRSP group, and 47% in the E4/DRSP group.

#### 14 **Thrombograms and TGA parameters**

##### 15 **Absolute values at baseline and after 6 cycles of treatment**

16 Analyses of thrombograms and resulting TGA parameters were performed on data from the 86 subjects in the final  
17 per protocol set population.

18 Thrombograms at baseline (n=86, entire baseline cohort) and after 6 cycles of treatment of women either treated  
19 with EE/LNG (n=24), EE/DRSP (n=28) or E4/DRSP (n=34) are shown in **Figure 3**. The mean thrombogram along with  
20 the 2.5<sup>th</sup>-97.5<sup>th</sup> percentile, is shown for the entire baseline cohort (n=86) and represents the reference interval.  
21 Mean [ $\pm$ 95%CI of the mean] thrombograms after 6 cycles of treatment are also presented. EE/LNG and EE/DRSP  
22 groups show a mean thrombogram [ $\pm$ 95%CI of the mean] outside the upper limit of the reference range, i.e., the  
23 97.5<sup>th</sup> percentile of all baseline thrombograms. On the other hand, the mean [ $\pm$ 95%CI of the mean] thrombogram  
24 of E4/DRSP is within this reference interval.



1 Mean values of each TGA parameter at baseline and at cycle 6 of the different treatment arms are reported in  
2 **Table 2**. Differences at baseline are observed depending on treatment arm: E4/DRSP arm has statistically higher  
3 peak (mean difference versus EE/LNG: 26.0 nM [95%CI: 2.1 to 49.9(5) nM] and versus EE/DRSP: 28.8(5) nM [95%CI:  
4 5.9(5) to 51.7(5) nM]), higher mVRI (mean difference versus EE/LNG: 16.5 nM/min [95%CI: 3.9 to 29.1 nM/min] and  
5 versus EE/DRSP: 19.4 nM/min [95%CI: 7.3 to 31.4 nM]) and shorter time to peak (mean difference versus EE/LNG:  
6 0.70 min [95%CI: 0.07 to 1.33 min] and versus EE/DRSP: 0.70 min [95%CI: 0.1 to 1.30 min]). After 6 cycles of  
7 treatment, no statistically significant difference is observed between EE/LNG and EE/DRSP groups ( $p$ -values  $>0.05$ ;  
8 **Figure 4**). On the other hand, the ETP, the Peak and the mVRI are significantly lower in the E4/DRSP group  
9 compared to EE/LNG and EE/DRSP while the Lag Time and the Time to peak are significantly higher in the E4/DRSP  
10 group compared to EE/DRSP, but not compared to EE/LNG. In addition, mean values of all TGA parameters are  
11 within the reference intervals (2.5<sup>th</sup> -97.5<sup>th</sup> percentile of entire baseline cohort) for E4/DRSP while mean ETP and  
12 peak height are out of ranges for both EE-containing products and the mean mVRI is also outside the reference  
13 range for EE/DRSP. As shown in **Table 3**, depending on the TGA parameter, up to 32% of values are outside the  
14 reference ranges for E4/DRSP while they reach 79% for EE/DRSP and 58% for EE/LNG.

#### 15 **Changes from baseline**

16 Compared to baseline, the ETP, the Peak and the mVRI are statistically different in all study arms after 6 cycles of  
17 treatment. The Lag time and the Time to peak are only statistically reduced in EE/LNG and EE/DRSP arms (**Figure 4**).  
18 These temporal parameters are not influenced by the intake of E4/DRSP. These data and the relative changes from  
19 baseline (%) for each TGA parameters are summarized in **Table 2** and **Figure 5**. All TGA parameters are statistically  
20 less impacted by E4/DRSP than EE/LNG or EE/DRSP. There is no statistical difference between EE/LNG and EE/DRSP.

#### 21 **DISCUSSION**

22 Hemostasis is a finely balanced physiological process and even if CHC-induced changes of coagulation factors often  
23 remain within the normal range of the population, the supra-additive effect tends to increase the total  
24 thrombogenicity. Hemostatic changes induced by CHCs involve levels of fibrinogen, prothrombin, FVII, FVIII, FIX, FX,  
25 FXII, AT, protein C, protein S, and TFPI (12). Over the past 30 years, many studies have been conducted to assess

1 the impact of the different CHCs on the coagulation system (12) and overall, these have revealed that the effect on  
2 hemostasis depends on the type of estrogens, their dose, and their association with progestins (5,7,21,34-36).

3 In this study, thrombin generation was assessed in a cohort of women treated during 6 cycles with either E4 in  
4 association with DRSP, EE in association with LNG or with DRSP. The global representation of the impact of these  
5 therapies on thrombin generation is illustrated by mean thrombograms on **Figure 3**. This simple visual analysis  
6 demonstrates the distinct impacts of these therapies on the global coagulation process.

7 Namely, the mean [ $\pm 95\%$ CI of the mean] thrombogram of women on E4/DRSP during 6 cycles does not exceed the  
8 reference baseline interval contrary to EE/LNG and EE/DRSP groups. This visual evaluation is confirmed by the  
9 quantitative analyses performed on the different TGA parameters, i.e., Lag time, Time to peak, Peak, ETP and mVRI  
10 (**Table 2**, **Table 3**). After 6 cycles, all TGA parameters were statistically less impacted in the E4/DRSP group  
11 compared to EE/LNG and EE/DRSP. Importantly, the changes from baseline were also always statistically smaller in  
12 the E4/DRSP group whatever the TGA parameter considered. On the other hand, no statistical difference was  
13 observed between EE/LNG and EE/DRSP although some parameters like the Lag time, the mVRI and to a lesser  
14 extend the Time to peak could be less impacted in the EE/LNG group compared to the EE/DRSP group but without  
15 reaching the significance level.

16 Thrombograms, however, revealed differences at baseline depending on treatment arm. Indeed, the mean  
17 thrombogram at baseline in the E4/DRSP group was shown to be higher for the Peak, the Time to peak and the  
18 mVRI parameters (**Table 2** and **Figure 4**). This observation is nevertheless mere coincidence as participants were  
19 first stratified by previous hormonal contraceptive use and by age, and then the assignment to one of the three  
20 treatment arms (i.e., E4/DRSP, EE/LNG or EE/DRSP) was performed using a computerized random allocation  
21 sequence. We searched for possible disproportion in the repartition of previous CHC users among the 3 treatment  
22 arms. No significant difference was observed between former users and non-users among each treatment arm, for  
23 all TGA parameters (p-value  $>0.05$ , unpaired t-tests) except for mVRI in the EE/DRSP group (p-value = 0.046). On  
24 the other hand, compared to EE/LNG and EE/DRSP, the mild observed hypercoagulable state at baseline, along with  
25 the smaller impact on thrombin generation after 6 months of treatment, reinforce the conclusion that E4 in  
26 association with DRSP induces less procoagulant changes than EE/LNG or EE/DRSP. This should be perceived as an

1 additional advantage of E4/DRSP over EE/LNG and EE/DRSP since the absolute thrombogram after 6 cycles is lower  
2 than the ones of EE/LNG and EE/DRSP even in a population showing a more procoagulant state at baseline.

3 Added to the results on the APC resistance<sup>1</sup>, these data mean that the global effect of an association of 15 mg E4  
4 with 3 mg DRSP has less impact on the entire coagulation than EE 30 mcg with LNG 150 mcg and EE 20 mcg with  
5 DRSP 3 mg. The mean thrombogram of the E4/DRSP group, along with all TGA parameters, standing within the  
6 reference ranges clearly delineates the neutral profile of this association on thrombin generation. Mean changes in  
7 ETP, Peak height, Lag time and Time to peak were below 20% while mVRI (a very sensitive TGA parameter) was only  
8 impacted by 24% whereas it is impacted by 105% and 144% in the EE/LNG and the EE/DRSP groups (**Table 2**).

9 Moreover, the different thrombograms along with TGA parameters between E4/DRSP and EE/DRSP strongly  
10 indicates that the lower impact of the new approved CHC, i.e., 15 mg E4/3 mg DRSP, is attributable to the  
11 difference in its estrogenic content and is not related to DRSP. Besides, the use of 4 mg DRSP alone revealed to be  
12 associated with a low impact on hemostasis parameters (37,38).

13 The fact that TGA parameters were not significantly influenced by the intake of E4/DRSP over the 6-month course  
14 of this study is very reassuring. The LETS study (Leiden Thrombophilia Study) revealed that patients with ETP above  
15 the 90<sup>th</sup> percentile on the control population were more prone to have a recurrent thrombotic event, suggesting a  
16 correlation between the risk of recurrent VTE and high ETP (23). In the combined LITE (Longitudinal Investigation of  
17 Thromboembolism Etiology) data, participants with Peak height values above the median were at 74% greater risk  
18 of VTE, as compared to those in the lowest quartile (24) suggesting that compounds with highest impact on  
19 thrombin generation are also those who are more prone to be associated with thromboembolic events. This is also  
20 supported by recent prediction models using the nAPCsr as surrogate marker for the risk of VTE (20). There is much  
21 other evidence that higher thrombin generation profiles, reflected by higher ETP, Peak height or mVRI and reduced  
22 Lag time or Time to peak, are associated with an increased risk of occurrence or recurrence of thrombosis (39-43).

23 Although a comparative phase IV study (i.e., post approval study) will be necessary to definitively prove the lower  
24 VTE risk profile of 15 mg E4/3 mg DRSP, the fact that no VTE event was observed in the US phase III study

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<sup>1</sup> These results demonstrated that the E4/DRSP combination has statistically less impact on the normalized APC sensitivity ratio (nAPCsr) than EE/LNG and EE/DRSP after 3 or 6 months of treatment.

1 evaluating E4/DRSP in 1864 women is already reassuring. Also 23% of the study participants had a BMI higher than  
2 30.0 kg/m<sup>2</sup>,(44) a well-known risk factor for VTE (45), suggesting a low incidence rate using this formulation.  
3 Previous studies of the same magnitude in similar populations using low risk CHCs (e.g. EE 10 mcg/norethindrone  
4 acetate 1 mg, a vaginal ring delivering EE 13 mcg and segesterone acetate 150 mcg per day or EE 30  
5 mcg/levonorgestrel 120 mcg) have reported higher absolute numbers of VTE events. Three thrombotic events were  
6 reported among 1683 (0.2%) US women using EE 10 mcg/norethindrone acetate 1 mg, of whom 18% were obese  
7 (46). Four VTE occurred among 1188 (0.3%) US women using a vaginal ring delivering EE 13 mcg and segesterone  
8 acetate 150 mcg per day (47) and 4 VTE occurred among 2031 (0.2%) US women, of whom 35% were obese, using a  
9 new contraceptive patch with dosing equivalent to an EE 30 mcg/levonorgestrel 120 mcg oral contraceptive pill  
10 (48). Thus, even with low-dose EE-containing CHCs, the thrombotic risk is still elevated substantially and higher  
11 than the one observed during the clinical development of E4/DRSP. This maybe in part explained by the unique  
12 mode of action of E4 which acts differently on the liver, potentially reducing the risk of VTE.

13 However, in the real-world (i.e. outside the setting of the clinical study in which exclusion criteria permit to select a  
14 low risk population) the inclusion of women with an unknown coagulopathy at the time of contraception initiation  
15 is unavoidable due to the current screening strategies. As previously described (13), the presence of a genetic  
16 mutation (i.e., FV Leiden, G20210A prothrombin mutations, protein C, protein S and antithrombin deficiencies)  
17 with CHCs leads to a synergistic and amplificative (rather than an additive) prothrombotic effect. Although  
18 contraindicated, cases of exposure in such population will occur and may inform on the amplificative effect of these  
19 prothrombotic conditions in presence of E4/DRSP. In a perspective, this could allow the computation of a synergy  
20 index for E4/DRSP, similar to what was done by Hugon-Rodin *et al.*(49) and Khialani *et al.* (50) with other CHC  
21 preparations. Finally, this will permit to appreciate the contribution of E4/DRSP on the global prothrombotic profile  
22 of a woman.

## 23 **CONCLUSION**

24 In conclusion, an association of 15 mg E4 with 3 mg DRSP does not have an impact on thrombin generation  
25 compared to EE-containing products which, either associated with LNG or DRSP, are able to increase the  
26 production of procoagulant factors and decrease the production of anticoagulant ones, shifting the patient to a

1 prothrombotic state. Ethinylestradiol-containing products thus generate prothrombotic environments contrary to  
2 E4 which demonstrates a neutral profile on hemostasis. Although this must be confirmed by data obtained from a  
3 post approval study, a previous experience with estradiol in association with nomegestrol acetate has permitted to  
4 validate the concept that these surrogate biomarkers may reflect the VTE profile of a specific CHC compared to a  
5 reference association.(51,52) This further suggests that E4/DRSP is less likely to be associated with VTE risk  
6 compared to EE-containing products.

#### 7 **ACKNOWLEDGEMENTS**

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9 study NCT02957630.

#### 10 **DATA AVAILABILITY**

11 Original data generated and analyzed during this study are included in this published article or in the data  
12 repositories listed in References.

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37



1 FIGURE LEGENDS

2  
3 **Figure 1. Representation of a thrombin generation curve and associated parameters which are provided by the**  
4 **software analyzing the thrombogram.**

5  
6 **Figure 2: Trial flow diagram**

7 *Abbreviations: DSRSP, drospirenone; EE, ethinylestradiol, E4, estetrol; LNG, levonorgestrel; n, number of subjects*

8  
9 **Figure 3: The mean [2.5<sup>th</sup> - 97.5<sup>th</sup> percentiles] thrombogram of the entire baseline cohort [N=87] and mean**  
10 **thrombograms [95%CI of the mean] after 6 cycles of treatment are presented.** The mean thrombogram of the  
11 entire baseline cohort is represented by the yellow line and the 2.5-97.5<sup>th</sup> percentile, indicating the reference  
12 ranges, are represented by yellow dotted lines. Women treated with a combination of ethinylestradiol and  
13 levonorgestrel are represented in blue, women treated with a combination of ethinylestradiol and drospirenone  
14 are represented in red and those treated with a combination of estetrol and drospirenone are represented in  
15 purple.

16  
17 **Figure 4. Graphical representation of all thrombin generation parameters at baseline and after 6 cycles of**  
18 **treatment for the different combined oral contraceptives associations.** Women treated with ethinylestradiol (EE)  
19 in association with levonorgestrel (LNG) are represented in blue, women treated with EE in association with  
20 drospirenone (DRSP) are in red and those treated with estetrol (E4) in association with DRSP are in purple.  
21 Differences between baseline, and cycle 6 for each treatment arm have been computed using paired t-test and  
22 differences between arms for a particular timepoint have been computed using ordinary ANOVA with Tukey's  
23 multiple comparison tests. \*, \*\*, \*\*\* and \*\*\*\* represent p-value  $\leq 0.05$ ,  $\leq 0.01$ ,  $\leq 0.001$  and  $< 0.0001$ , respectively.  
24 Only differences which are statistically significant are reported.

25 *Abbreviations: DRSP, drospirenone; ETP, endogenous thrombin potential, EE, ethinylestradiol; E4, estetrol; LNG,*  
26 *levonorgestrel, mVRI, mean velocity rate index.*

27  
28 **Figure 5: Change from baseline (%) of all thrombin generation parameters after 6 cycles of treatment for the**  
29 **different combined oral contraceptives associations.** Women treated with ethinylestradiol (EE) in association with  
30 levonorgestrel (LNG) are represented in blue, women treated with EE in association with drospirenone (DRSP) are  
31 in red and those treated with estetrol (E4) in association with DRSP are in purple. Differences between arms have  
32 been computed using one way ANOVA with Tukey's multiple comparison tests. \*, \*\*, \*\*\* and \*\*\*\* represent p-  
33 value  $\leq 0.05$ ,  $\leq 0.01$ ,  $\leq 0.001$  and  $< 0.0001$ , respectively. Only differences which are statistically significant are  
34 reported.

35 *Abbreviations: DRSP, drospirenone; ETP, endogenous thrombin potential, EE, ethinylestradiol; E4, estetrol; LNG,*  
36 *levonorgestrel, mVRI, mean velocity rate index.*

1 **Table 1: Mean demographic data at study entry**

2  
3 *Abbreviations: BMI, body mass index; DRSP, drospirenone; EE, ethinylestradiol; E4, estetrol; LNG, levonorgestrel; n, number of*  
4 *subjects*

	<b>15 mg E4 - 3 mg DRSP N = 38</b>	<b>30 µg EE - 150 µg LNG N = 29</b>	<b>20 µg EE - 3 mg DRSP N = 31</b>	<b>All N = 98</b>
<b>Age, y (range)</b>	26.7 (19 – 47)	26.2 (18 – 44)	25.6 (18 – 40)	26.2 (18 – 47)
<b>Weight, kg (range)</b>	68.1 (53.1 – 97.8)	65.6 (50.4 – 79.2)	63.2 (50.3 – 80.7)	65.8 (50.3 – 97.8)
<b>Height, cm (range)</b>	170.8 (159 – 188)	169.6 (160 – 181)	168.4 (155 – 183)	169.7 (155 – 188)
<b>BMI, kg/m<sup>2</sup> (range)</b>	23.33 (19.2 – 30.0)	22.83 (18.3 – 29.8)	22.27 (18.6 – 26.7)	22.85 (18.3 – 30.0)

5

1 **Table 2: Thrombin generation parameters with the different combined oral contraceptive associations at baseline and cycle 6. Mean values ± standard deviations are shown**

2  
3 *Abbreviations: DRSP, drospirenone; EE, ethinylestradiol; E4, estetrol; ETP, endogenous thrombin potential; LNG, levonorgestrel; mVRI, mean velocity rate index*

	ETP (nM.min)				Peak height (nM)				Lag time (min)				Time to peak (min)				mVRI (nM/min)			
	Baseline	Cycle 6	Relative diff. (%)	P-value	Baseline	Cycle 6	Relative diff. (%)	P-value	Baseline	Cycle 6	Relative diff. (%)	P-value	Baseline	Cycle 6	Relative diff. (%)	P-value	Baseline	Cycle 6	Relative diff. (%)	P-value
EE/LNG	1219 ± 180	1588 ± 204	32.0 ± 20.5	<0.0001	192.5 ± 39.6	287.3 ± 43.7	53.6 ± 29.4	<0.0001	3.01 ± 0.51	2.65(5) ± 0.40	-10.5 ± 12.9(5)	0.0005	6.72 ± 1.28	5.47 ± 0.91	-18.0 ± 8.5	<0.0001	55.6 ± 19.0	107.1 ± 29.6	105.0 ± 56.4	<0.0001
EE/DRSP	1226 ± 146	1613 ± 197	32.8 ± 19.0	<0.0001	189.7 ± 33.8	304.3 ± 35.6	65.5 ± 38.4	<0.0001	2.97 ± 0.43	2.49 ± 0.29	-15.2(5) ± 10.4	<0.0001	6.72 ± 0.91	5.13 ± 0.58	-23.0 ± 8.1	<0.0001	52.8 ± 15.8	118.5 ± 24.9	143.6 ± 98.8	<0.0001
E4/DRSP	1257 ± 150	1456 ± 186	16.8 ± 15.9	<0.0001	218.5 ± 39.0	253.2 ± 42.6	17.3 ± 16.5	<0.0001	2.83 ± 0.39	2.72 ± 0.32	-2.4(5) ± 15.8	0.1632	6.02 ± 0.80	5.79 ± 0.78	-3.2 ± 12.2	0.0747	72.2 ± 23.0	87.2 ± 28.5	24.3 ± 27.9	<0.0001
P-value†	0.6167	0.0042	0.0010	/	0.0054	<0.0001	<0.0001	/	0.2636	0.0275	0.0013	/	0.0078	0.0046	<0.0001		0.0004	0.0001	<0.0001	/

4 † P-value had been estimated by using an ordinary one-way ANOVA. A Tukey's multiple comparisons test, with a single pooled variance has been run to assess the difference between the groups. P-value < 0.05 are considered statistically  
5 significant.  
6  
7

1 **Table 3: Reference intervals ([2.5<sup>th</sup> – 97.5<sup>th</sup> percentile] of entire baseline cohort) of thrombin generation**  
 2 **parameters and out of ranges results after 6 cycles of treatment.**

3  
 4 *Abbreviations: DRSP, drospirenone; EE, ethinylestradiol; E4, estetrol; ETP, endogenous thrombin potential; LNG,*  
 5 *levonorgestrel; mVRI, mean velocity rate index*

	<b>ETP (nM.min)</b>	<b>Peak Height (nM)</b>	<b>Lag time (min)</b>	<b>Time to peak (min)</b>	<b>mVRI(nM/min)</b>
Reference ranges	906 – 1562	119.6 -282.0	2.09 – 3.80	5.00 – 8.64	24.8 – 111.0
<b>EE/LNG (N=25)</b>	10(42%)	14 (58%)	0 (0%)	6 (25%)	10 (42%)
<b>EE/DRSP (N=28)</b>	18 (64%)	22 (79%)	2 (7%)	12 (43%)	19 (68%)
<b>E4/DRSP (N=34)</b>	11 (32%)	9 (26%)	0 (0%)	5 (15%)	9 (26%)

6  
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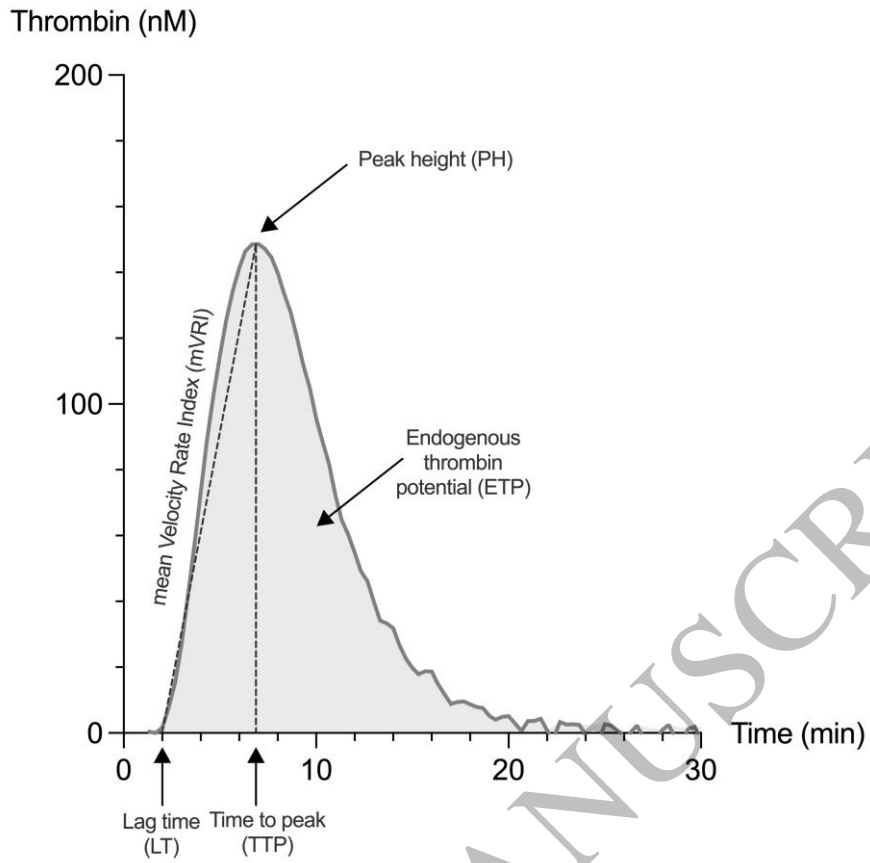


Figure 1  
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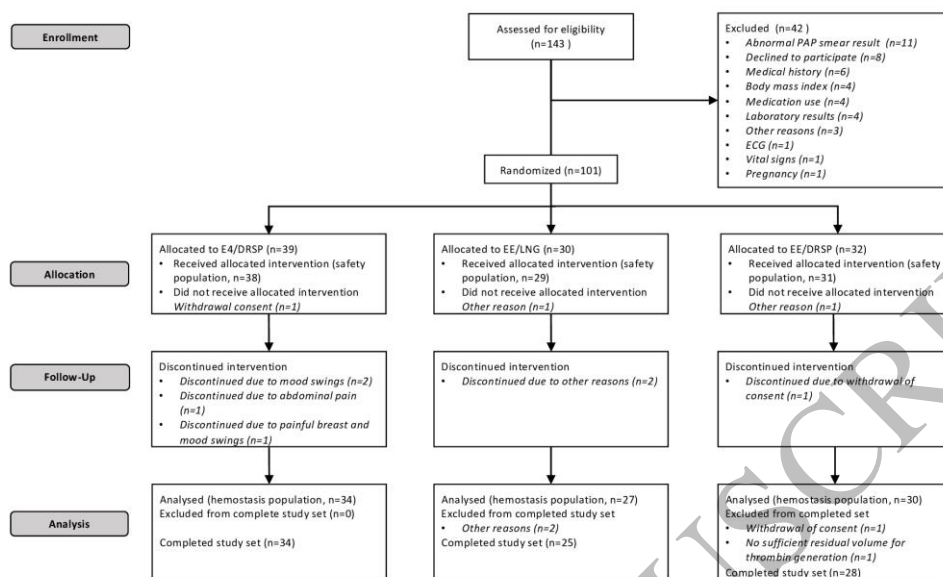
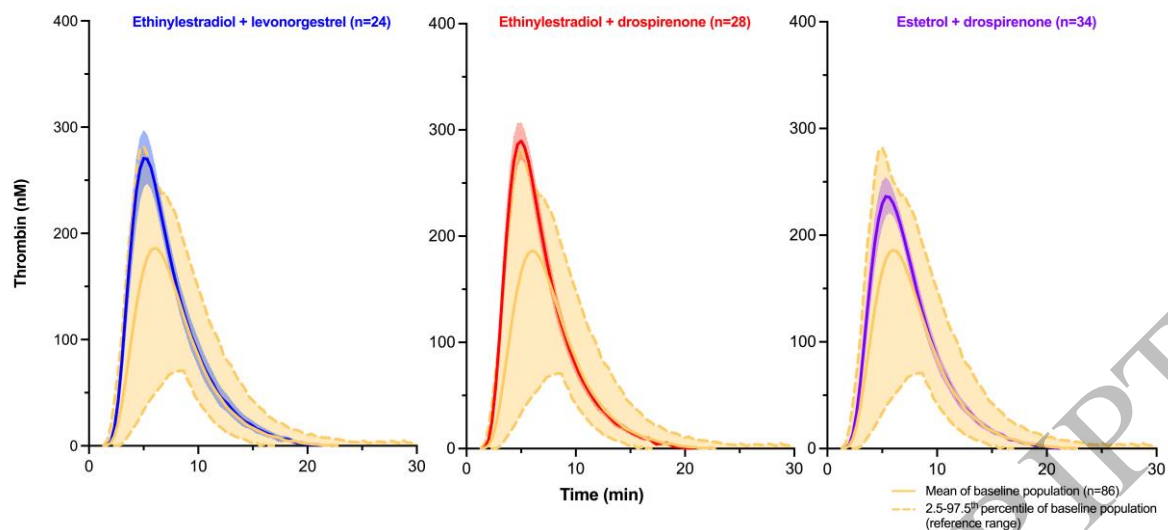


Figure 2  
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**Figure 3**  
160x72 mm (.75 x DPI)

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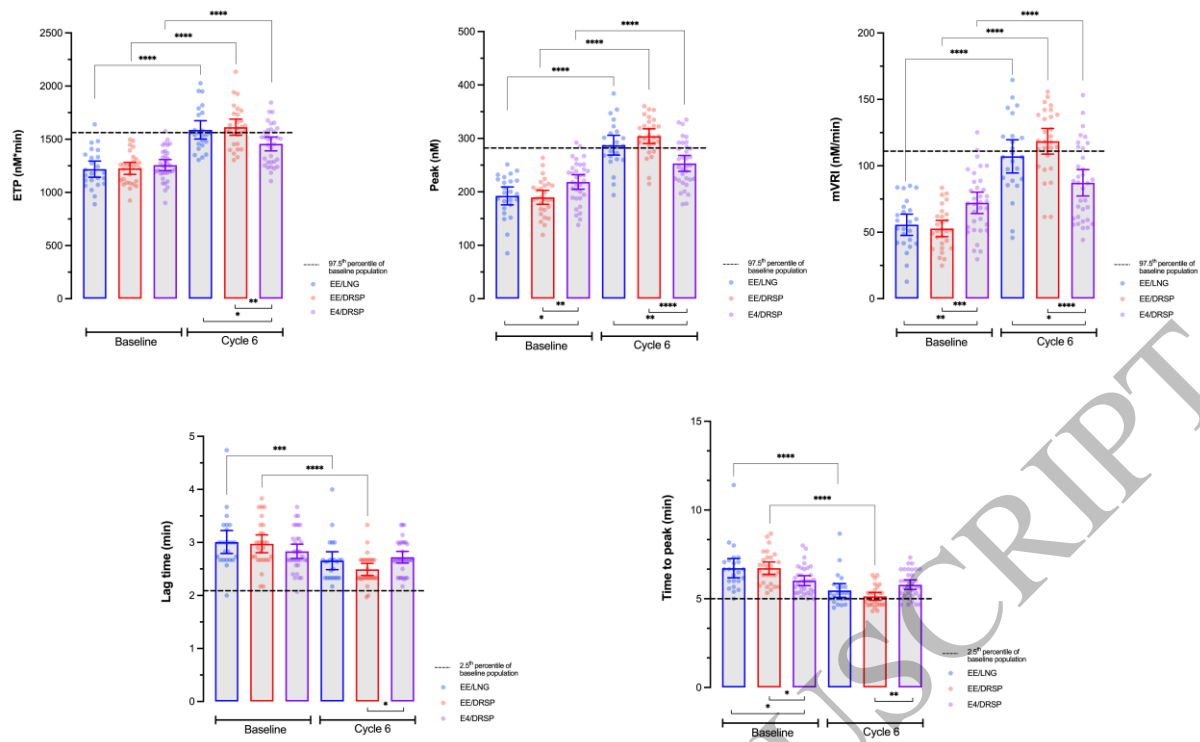


Figure 4  
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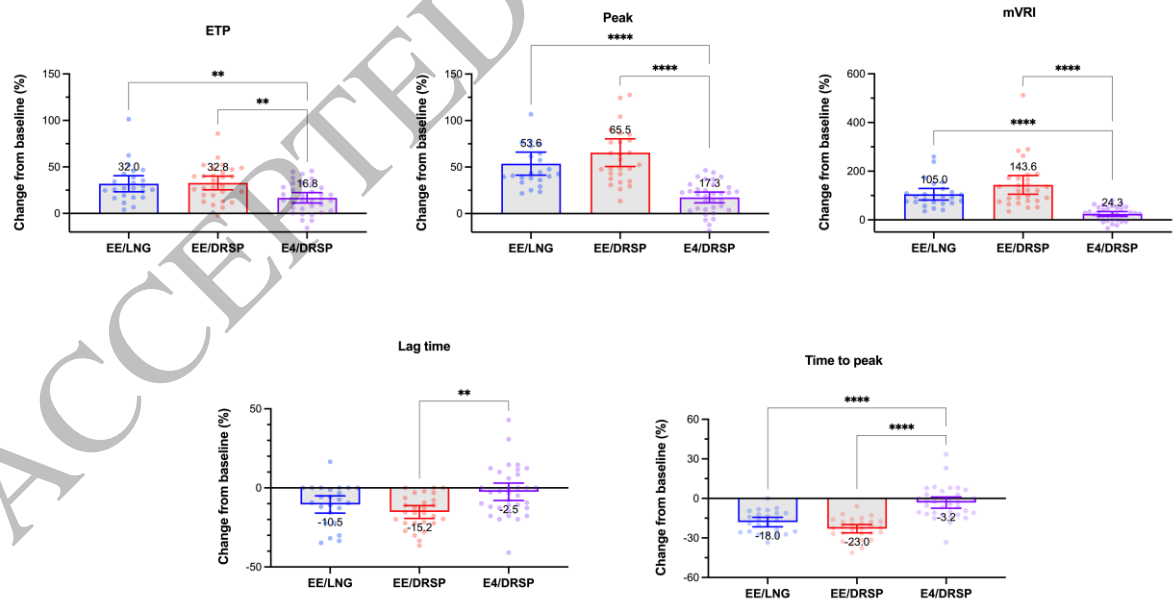


Figure 5  
160x82 mm (.75 x DPI)

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