Hormonal contraceptives : effectiveness and adverse effects

Vliet, H.A.A.M. van

Chapter 1

General Introduction

Oral contraceptives and adverse effects

In 1959 the first contraceptive pill composed of 9.85 mg norethynodrel and 150 µg mestranol (Enovid®, G.D. Searle&Co., Chicago, USA) was introduced on the market. Because of the high effectiveness in preventing pregnancy and ease of use, oral contraceptives soon became a popular contraceptive method. Nowadays, more than 100 million women worldwide are using contraceptive pills [1]. However, like most prescriptions, oral contraceptives also have side effects. Due to the large number of women taking oral contraceptives even a low risk of side effects will affect many women. The aim of this thesis was to balance effectiveness with adverse effects of hormonal contraceptives.

Shortly after the introduction of the contraceptive pill case reports describing venous thrombosis, myocardial infarction and stroke in women using oral contraceptives appeared [2-4]. Since the absolute risk of cardiovascular disease in women of reproductive age is very low, e.g. one per 10.000 women per year for venous thrombosis, evaluation of the risk of cardiovascular events in a randomized controlled trial is practically impossible [5]. The best evidence needs to come from observational studies like cohort studies and case-control studies. In case of adverse effects these studies provide data that are as valid as evidence from randomized controlled trials [6,7]. Observational studies showed a 2- to 6-fold increased risk of venous thrombosis, a 2- to 5-fold increased risk of myocardial infarction, a 2- to 5-fold increased risk of stroke and a 4-fold increased risk of peripheral artery disease in users of oral contraceptives compared to women not using oral contraceptives [5,8-11].

Women carrying hereditary clotting defects, such as the factor V Leiden mutation, prothrombin 20210A mutation, deficiencies of protein C, protein S or antithrombin were found to be at much higher risk of venous thrombosis when they use oral contraceptives [5,8,9]. The presence of the factor V Leiden mutation enhances the risk of thrombosis by a factor 4 to 10 in heterozygotes and by a factor of 50 to 100 in homozygotes. Heterozygous carriers of the factor V Leiden mutation were reported to have an 35-fold increased risk of thrombosis when they use oral contraceptives and homozygous carriership was found to confer even a higher risk [12-14]. The synergistic effect of clotting defects with pill use on the risk of thrombosis was also observed for women carrying the prothrombin 20210A mutation [15]. This mutation increases the risk of thrombosis by a factor 2. Carriers of the prothrombin 20210A mutation were found to have a 16-fold increased risk of thrombosis when they use oral contraceptives [15]. Definite data on the risk of thrombosis in women with deficiencies of protein C, protein S or antithrombin who use oral contraceptives are lacking due to the low prevalence of these clotting defects but the thrombotic risk seems to be increased [16-18].

Minor adverse effects associated with combined oral contraceptive use include bleeding disturbances, nausea, vomiting, headache, breast tenderness, weight gain and loss of libido [19]. These minor adverse effects may discourage compliance with and continuation of oral contraceptive use resulting in an increased risk of unintended pregnancies [20-22]. Since

minor adverse events are common in oral contraceptive users randomized comparisons are feasible and preferred.

In order to reduce the unwanted effects of oral contraceptives four approaches have been used: (i) lowering of the steroid dose; (ii) development of new formulas and schedules of administration; (iii) development of new steroids and (iv) development of new routes of administration.

Reduction of steroid dose

In the first decennia after the introduction of the pill the estrogen dose was gradually lowered from 150 µg, to 80 µg, to 50 µg in the 1960s and to 35-30 µg and 20 µg in the 1970s [23]. Subsequent studies showed that the thrombotic risk of oral contraceptives is positively associated with the estrogen dose in oral contraceptives. Contraceptive pills containing 100-150 µg of estrogen were found to have a higher risk of thrombosis than contraceptive pills containing 50 µg of estrogen [24-26]. Similarly, it was shown that 50 µg estrogen oral contraceptives are associated with a higher thrombotic risk than 30 µg estrogen oral contraceptives [9,27,28]. Recently, studies found a higher risk of thrombosis for pills containing 30 µg of estrogen compared to pills containing 20 µg of estrogen [29,30].

Following the reduction of estrogen in oral contraceptives, the dose of progestogen was also decreased in recent decades [31-33]. When focussing on the amount of progestogen in oral contraceptives, it is necessary to consider both dose and potency since each progestogen has its own unique biologic and pharmacologic activity.

New formulas and schedules of administration

Initially oral contraceptives contained a fixed dose of estrogen and progesterone for 21 days, so called monophasic preparations. In an attempt to reduce the steroid dose further, while maintaining adequate contraceptive effectiveness and cycle control, biphasic and triphasic oral contraceptives were developed in the 1970s and 80s [19]. These preparations consist of two or three phases, each with a different progesterone dosage and in some preparations estrogen dosage. In the first phase progestogen levels are low, followed by a higher dose of the steroids in the second and third phase.

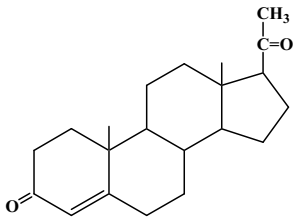
Despite the publication of numerous randomized controlled trials comparing triphasic or biphasic oral contraceptives with monophasic oral contraceptives in the past years, it is unknown whether the multiphasic approach differ from the monophasic approach in terms of contraceptive effectiveness, cycle control, minor adverse effects and discontinuation. The reasons for this dearth of knowledge lie in the differences in formulations of the studied pills and the variable methods of collecting and analyzing bleeding data making comparisons troublesome as well as the small sample sizes of the studies. Nevertheless, in an attempt to obtain some insight in how the multiphasic approach compares itself with the monophasic approach, we conducted a systematic review of randomized controlled trials

comparing triphasic pills with monophasic pills and performed a meta-analysis for the outcomes contraceptive efficacy, bleeding pattern and discontinuation in **chapter 2**. In **chapter 3** we systematically reviewed the literature for trials comparing biphasic preparations with monophasic preparations. **Chapter 4** is a systematic review of randomized controlled trials comparing triphasic oral contraceptives with biphasic oral contraceptives.

The main reason for conducting systematic reviews based on randomized controlled trials is to minimize bias [34]. Since controlled clinical trials of poor methodological quality tend to overestimate treatment effects by 30 to 50%, quality assessment of trials within systematic reviews is important [35]. In **chapter 5** we investigate the frequency of quality assessment of randomized controlled trials within systematic reviews and the incorporation of the quality assessment in the analysis.

New steroids

The early contraceptive formulations in the 1960s contained norethynodrel, norethisterone and lynestrenol as progestogen content (Figure 1 and 2). These progestogens are derived from the estrane steroids and are also known as first-generation progestogens [36]. In the 1970s the second-generation progestogens norgestrel and levonorgestrel were introduced (Figure 3). Second-generations progestogens stem from the gonane structure [36]. The third-generation progestogens gestodene and desogestrel, which are also constructed out of the gonane steroids, were marketed from the 1980s (Figure 3) [36]. Based on the structural formula norgestimate is classified as a third-generation progesterone (Figure 3). However, after uptake norgestimate is in part, rapidly converted to levenorgestrel so metabolically it may belong more to the second-generation progestogens [37]. Two progestogens are not covered by the classification system in generations, cyproterone acetate which is derived from the pregnane structure and drospirenone which is derived from 17α-spironolactone (Figure 4 and 5).



PROGESTERONE

Figure 1. Chemical structure of progesterone.
The figure was kindly provided by Professor Frank Z. Stanczyk, Department of Obstetrics and Gynecology, Keck School of Medicine, University of Southern California, Los Angeles.

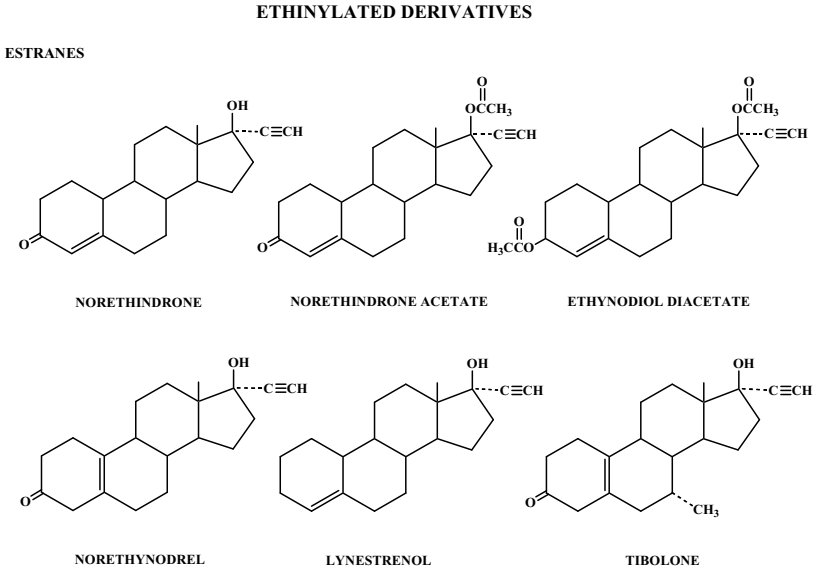


Figure 2. Chemical structures of the first-generation progestogens norethynodrel, norethisterone and lynestrenol.
The figure was kindly provided by Professor Frank Z. Stanczyk, Department of Obstetrics and Gynecology, Keck School of Medicine, University of Southern California, Los Angeles.

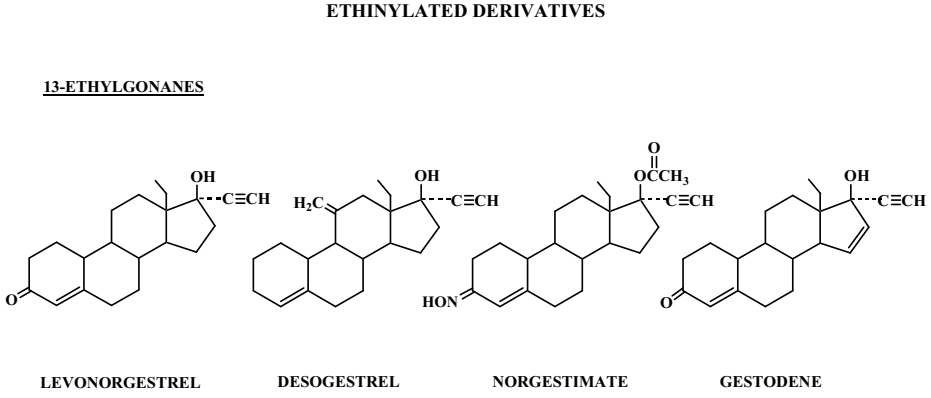


Figure 3. Chemical structures of the second-generation progestogen levonorgestrel and the third-generation progestogens desogestrel, gestodene and norgestimate.
The figure was kindly provided by Professor Frank Z. Stanczyk, Department of Obstetrics and Gynecology, Keck School of Medicine, University of Southern California, Los Angeles.

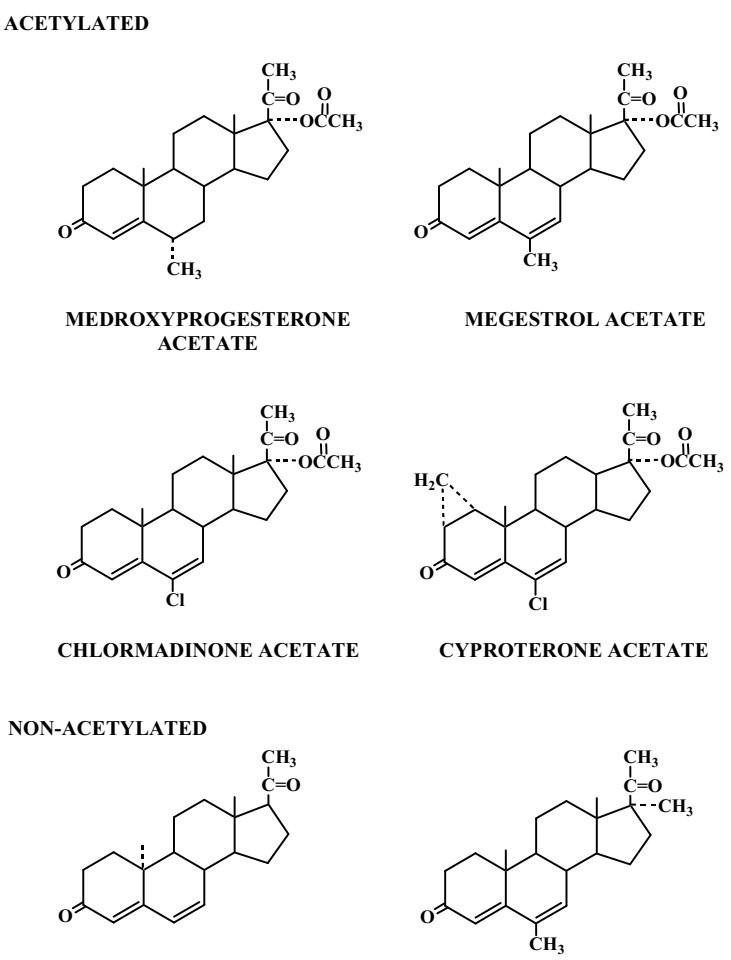


Figure 4. Chemical structure of the progestogen cyproterone acetate.
The figure was kindly provided by Professor Frank Z. Stanczyk, Department of Obstetrics and Gynecology, Keck School of Medicine, University of Southern California, Los Angeles.

The third-generation progestogens gestodene and desogestrel were originally developed to reduce the deleterious arterial adverse effects of oral contraceptives [19]. They were less androgenic than second-generation progestogens and seemed to have a favourable effect on lipid and carbohydrate metabolism [19,37-39]. Yet, in 1995 three studies observed a 2-fold increased risk of venous thrombosis in women using combined oral contraceptives containing the third-generation progestogens gestodene and desogestrel compared to women using second-generation combined oral contraceptives [40-42]. These findings led to a heated debate. Several studies confirmed the increased risk of venous thrombosis of third-generation oral contraceptives [5,8,43-45]. Other reports did not observe a difference in risk between

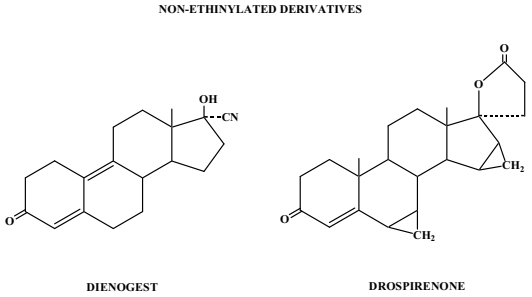


Figure 5. Chemical structure of the progestogen drospirenone.
The figure was kindly provided by Professor Frank Z. Stanczyk, Department of Obstetrics and Gynecology, Keck School of Medicine, University of Southern California, Los Angeles.

second- and third-generation oral contraceptives and/or explained the difference in risk by confounding and bias [8,45-47]. A meta-analysis by Kemmeren et al., combining the various studies, demonstrated a 1.7-fold (95% CI 1.4 to 2.0) increased risk of venous thrombosis for third-generation oral contraceptives compared to second-generation oral contraceptives [48]. Interestingly, sub-analyses showed that the odds ratio for third-generation oral contraceptives versus second-generation oral contraceptives for studies sponsored by the pharmaceutical industry was 1.3 (1.0-1.7) compared to 2.3 (1.7 to 3.2) for studies that were financed with public funds or through charities. All arguments that the differences in risk were caused by confounding and bias were invalidated by reasoning or by new analyses [8,45,48]. In 1999 combined oral contraceptives containing the progestogen cyproterone acetate were found to confer even a higher thrombotic risk, 4-fold compared to combined oral contraceptives containing levonorgestrel [49].

At the time of the debate on the thrombotic risk of third-generation oral contraceptives a biological explanation for the increased risk of thrombosis associated with oral contraceptive use and the differences in thrombotic risk between second-generation and third-generation oral contraceptives was lacking. Numerous studies had assessed the effect of the pill on the hemostatic system and many clotting factors were found to be influenced [11,50-52]. Observed prothrombotic effects of combined oral contraceptives included increased levels of the procoagulant factors prothrombin, factor VII, VIII, IX, X, XI, XII, von Willebrand factor and fibrinogen and decreased levels of the anticoagulant factors protein S and antithrombin. Reported effects of the pill in an antithrombotic direction included increased levels of the anticoagulant factor protein C and α-antitrypsin and changes in fibrinolytic factors e.g. plasminogen and plasminogen activator inhibitor. Because the net effect of the changes in the different haemostatic systems was not known, the interpretation of the observed effects varied between researchers. Differential effects on coagulation and fibrinolysis parameters between second-generation and third-generation oral contraceptives were not observed or not consistent [53].

A breakthrough in the biological understanding of the increased risk of thrombosis associated with oral contraceptive use and the difference in thrombotic risk between second-generation and third-generation oral contraceptives was the observation that oral contraceptives induce an acquired form of APC-resistance [54,55]. APC-resistance or a poor anticoagulant response of plasma to activated protein C was first reported by Dählback in a family with a hereditary tendency to venous thrombosis and appeared to be an important risk factor for venous thrombosis [56-58]. A year later, Bertina and co-workers discovered the factor V Leiden mutation which was found to be the most common cause of hereditary APC-resistance [59]. The mutation in the gene of factor V results in the replacement of an amino acid (Arg506 by Gln) at a cleavage site of APC in activated factor V which makes activated factor V less susceptible to inactivation by APC. Oral contraceptive use and pregnancy were found to induce an acquired form of APC-resistance [54,55,60-62]. APC-resistance, not caused by the factor V Leiden mutation, was reported to be a risk factor for venous thrombosis [63,64].

After the observations by Dählback several tests for the detection of APC-resistance were developed. The original or aPTT-based APC-resistance test measures the effect of APC on the clotting time of plasma in which coagulation is initiated via the intrinsic pathway [65]. The research group of Rosing and Hemker developed a thrombin generation-based APC-resistance test which quantifies the effect of APC on the time integral of thrombin formation in plasma in which coagulation is initiated via the extrinsic coagulation pathway [66,67]. Women using oral contraceptives were found to be resistant to APC by this thrombin generation-based APC-resistance test but more interestingly women using third-generation oral contraceptives were found to be more resistant to APC than women using second-generation oral contraceptives [68,69]. This difference in APC-resistance yielded an explanation for the differences in thrombotic risk between second-generation and third-generation oral contraceptives. The thrombin-generation based APC-resistance test was validated in the in the Leiden Thrombophilia Study and showed to predict the risk of venous thrombosis in users of oral contraceptives, as well as in non-users and men [70].

The thrombin-generation based APC-resistance test is a global assay which incorporates the effects on individual clotting factors and combines them into a net effect. After the observed differences in APC-resistance, studies also showed a different effect of second- and third-generation oral contraceptives on several clotting factors. Users of oral contraceptives containing desogestrel were found to have higher levels of the procoagulant factor prothrombin and lower levels of the anticoagulant factor protein S which are both risk factors for venous thrombosis [71-73]. Other observed differences included higher levels of the procoagulant factor VII and antifibrinolytic factor TAFI and lower levels of factor V in women using desogestrel-containing oral contraceptives compared to levonorgestrel-containing oral contraceptives [71,74,75].

In 2000 a new combined oral contraceptive containing 3 mg of the progestogen drospirenone and 30 µg ethinylestradiol was brought on the market (Yasmin®, Bayer Schering

Pharma, Berlin, Germany). Due to the antimineralocorticoid and antiandrogenic properties of drospirenone, this contraceptive pill would theoretically not lead to weight gain and would have a beneficial effect on acne, seborrhoea and hirsutism [76,77]. Three years after the introduction of the drospirenone-containing oral contraceptive concern was raised about the thrombotic safety of this pill [78,79]. Although already widely used no studies on the risk of venous thrombosis of the drospirenone-containing oral contraceptive were available. In order to predict the thrombotic risk of oral contraceptives containing drospirenone we compare in **chapter 6** the thrombin generation-based APC-resistance in users of oral contraceptives containing drospirenone with the APC-resistance in users of oral contraceptives containing second-generation progestogens, third-generation progestogens and cyproterone acetate.

As mentioned above the thrombin generation-based APC-resistance test is a global test which is influenced by several procoagulant and anticoagulant factors [80]. The two main determinants of the test are free protein S and free tissue factor pathway inhibitor (TFPI) [80,81]. Hereditary deficiencies of protein S and low plasma levels of TFPI have been associated with an increased risk of thrombosis [82-84]. To gain more insight in the biological basis of acquired APC-resistance in oral contraceptive users and the different effects of various types of oral contraceptives on APC-resistance, we study in **chapter 7** the resistance to APC, free and total protein S and TFPI activity, total antigen and free antigen in users of oral contraceptives containing different progestogens.

Due to the low incidence of venous thrombosis in users of hormonal contraceptives, assessment of the thrombotic risk of a new preparation requires hundreds of thousands of participants [5]. This sample size renders a clinical study before market authorization virtually unfeasible. In search for a marker for the thrombotic risk of a hormonal contraceptive, Odlind et al. performed a literature study which evaluated whether the effect of an oral contraceptive on Sex Hormone Binding Globulin (SHBG) levels could be an indicator for the risk of thrombosis [85]. SHBG is produced in the liver and is a carrier protein for estrogen and testosterone [86]. In women estrogens cause a dose-related increase in SHBG, whereas progestogens induce a decrease of SHBG, the extent of which is depending on both dose and type of progestogen [86-89]. The type-related differences in progestogen-induced decrease of SHBG might be interpreted as differences in anti-estrogenic properties of progestogens. Thus, the effect of an oral contraceptive on SHBG levels can be seen as the sum of the estrogenic effect of ethinylestradiol and the anti-estrogenic effect of the progestogen resulting in the total *estrogenicity* of the pill [85,90].

The literature study by Odlind et al. demonstrated a relationship between the known thrombotic risk of second-generation, third-generation and cyproterone acetate containing oral contraceptives and the effect of the various types of oral contraceptives on SHBG [85]. In agreement with the increased risk of thrombosis gestodene- and desogestrel-containing contraceptive pills were found more estrogenic, i.e. increased SHBG more,

than levonorgestrel-containing pills [85]. Oral contraceptives containing cyproterone acetate were associated with the highest SHBG levels [85]. A randomized controlled trial comparing SHBG levels in women using desogestrel-containing oral contraceptives and women using levonorgestrel-containing oral contraceptives confirmed the higher levels of SHBG in desogestrel-containing pill users and an association between SHBG levels and the resistance to APC measured with the classical aPTT-based APC-resistance test was reported [91]. To test the usefulness of SHBG as a marker for the thrombotic risk of an oral contraceptive we compare in **chapter 8** the plasma levels of SHBG and the resistance to APC determined with a thrombin generation-based APC-resistance test in users of oral contraceptives containing either second- or third-generation progestogens, drospirenone or cyproterone acetate.

New routes of administration

Contraceptive vaginal ring and transdermal patch

Recently two new contraceptive formulations using alternative administration routes of combined steroids were introduced, the vaginal ring (NuvaRing®, Organon, Oss, The Netherlands) and the transdermal patch (Ortho-Evra®/Evra®, Ortho-McNeil-Janssen Pharmaceuticals, Raritan, NJ, USA). The rationale of their development was to improve convenience and increase compliance by avoiding daily contraceptive taking and to reduce the doses of contraceptive steroids [92-95]. NuvaRing® is a flexible, soft ring measuring 5.4cm in diameter and 4mm thickness which can easily be inserted into and removed from the vagina by the woman herself [96,97]. The ring steadily releases 120 µg of etonogestrel and 15 µg of ethinylestradiol per day over 3 consecutive weeks [96,97]. Etonogestrel or 3-keto desogestrel is the biologically active form of the third-generation progestogen desogestrel [37]. Ortho-Evra®/Evra® is a thin adhesive square measuring 20cm^2 which is applied on the skin [93,98]. The patch continuously delivers a daily dose of 150 µg norelgestromin and 20 µg ethinylestradiol for 1 week [93,98]. Norelgestromin is the active metabolite of the progestogen norgestimate.

Both methods provide a sustained release of contraceptive steroids to the systemic circulation which results in constant plasma levels and avoids the daily peaks and troughs that are seen with oral contraceptive use [93,94,99]. Loss of bioavailability, due to gastrointestinal degradation and first-pass hepatic metabolism, is avoided by direct systemic administration allowing lower dosages of hormones to be used [93,94,96].

In women using the contraceptive ring peak serum concentrations of ethinylestradiol are 70 percent lower than in women using an oral contraceptive composed of 150 µg desogestrel and 30 µg ethinylestradiol [100]. Analysis of area under the ethinylestradiol concentration-versus-time curve showed that exposure to ethinylestradiol during use of the contraceptive ring is 2 times lower than during use of a 150 µg levonorgestrel and 30 µg ethinylestradiol oral contraceptive [99]. Maximum serum concentrations of etonorgestrel in women using the

contraceptive ring are 60 percent lower than in women using an oral contraceptive containing 150 µg desogestrel and 30 µg ethinylestradiol [100].

During use of the contraceptive patch exposure to ethinylestradiol as measured by the area under the curve and steady state concentration is 60 percent higher than with use of an oral contraceptive composed of 250 µg norgestimate and 35 µg ethinylestradiol [98,101]. Peak ethinylestradiol concentrations in women using the contraceptive patch are 25 percent lower than in women using a 250 µg norgestimate and 35 µg ethinylestradiol contraceptive pill [98,101]. Norelgestromin exposure is similar during use of the contraceptive patch and pill [101].

Limited data are available on the thrombotic safety of the contraceptive ring and contraceptive patch. No studies have been published on the risk of venous thrombosis of the contraceptive ring and conflicting results have been reported on the thrombotic risk of the contraceptive patch. A nested case-control by Jick et al. found no difference in the risk of thrombosis between the contraceptive patch and norgestimate-containing oral contraceptives [102]. However, a cohort and nested case-control study by Cole et al. observed a 2-fold increased risk of thrombosis for users of the contraceptive patch compared to users of oral contraceptives containing norgestimate [103]. In order to predict the thrombotic risk of the contraceptive ring and the contraceptive patch we compare in **chapter 9** the thrombin generation-based APC-resistance and SHBG levels in users of the contraceptive ring, the contraceptive patch and an oral contraceptive containing 150 µg levonorgestrel and 30 µg ethinylestradiol.

Intrauterine system

From the 1990s onwards the levonorgestrel-releasing intrauterine system (Mirena®, Bayer Schering Pharma, Berlin, Germany) was marketed. The levonorgestrel-intrauterine system was developed to reduce the increase in amount and duration of menstrual blood loss and to diminish the increase in episodes of intermenstrual bleeding as common side effects of intrauterine devices [104,105]. Other adverse effects associated with the use of intrauterine devices include abdominal pain, pelvic inflammatory disease and perforation of the uterus [105].

The levonorgestrel-intrauterine system is a 32mm long T-shaped plastic device with a reservoir around the vertical stem containing 52 mg levonorgestrel [106,107]. The system is inserted in the uterine cavity where it continuously releases 14 to 20 µg levonorgestrel per 24 hours [106,107]. The recommended duration of use of the device is 5 years. Following insertion, plasma levels of levonorgestrel are 150 to 200 µg/mL in the peripheral blood [106,108]. In comparison, maximal levels during use of a 30 µg levonorgestrel-only pill are 800 pg/mL [109].

No studies have been published on the thrombotic safety of the levonorgestrel-intrauterine system. Little is also known about the thrombotic risk of other progestogen-only contraceptives. Three case-control studies assessed the risk of thrombosis for users of progestogen-only pills or injectables and suggested that there is little or no increased risk of thrombosis

[110-112]. However, these studies are limited by the small number of women using these types of contraceptives that were included. In **chapter 10** we evaluate the thrombotic risk of the levonorgestrel-intrauterine system by comparing the thrombin generation-based APC-resistance of plasma from women before and after insertion of a levonorgestrel-releasing or a copper-containing intrauterine device.

Natural cycle

As mentioned above elevated levels of estradiol and progesterone, e.g. during pregnancy or ovarian stimulation, as well as exogenously administered estrogens and progestogens during oral contraceptive use and hormone replacement therapy, were shown to induce an acquired form of APC- resistance [60,61,69,81,113-117]. Throughout the natural menstrual cycle concentrations of estradiol and progesterone vary. Briefly, at the early follicular phase estradiol and progesterone levels are low, during the follicular phase estrogen levels increase with the highest levels prior to the LH-surge, while after the LH-surge estrogen levels decrease and progesterone levels increase with the hightest levels mid-luteal [118]. Several clotting factors were also found to display a cyclic pattern. Levels of fibrinogen and von Willebrand factor were reported to be highest in the luteal phase [119-121]. Maximum levels of factor VIII were shown around ovulation [122]. A reduction in the concentration of activated factor VII was observed during the follicular phase [123]. Plasminogen activator inhibitor-1 (PAI-1) and D-dimer were found to decrease during the luteal phase [124,125]. In **chapter 11** we evaluated whether varying levels of estradiol and progesterone during the menstrual cycle are associated with differences in APC-resistance determined with the thrombin generation-based APC-resistance test.

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