RESIDUAL OVARIAN ACTIVITY DURING ORAL CONTRACEPTION

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS

PROF.DR.IR. J.H. van BEMMEL

EN VOLGENS BESLUIT VAN HET COLLEGE VOOR PROMOTIES

DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 5 FEBRUARI 2003 OM 15.45 UUR
DOOR
ARNE MARIUS VAN HEUSDEN
Geboren te BILTHOVEN

The work presented in this thesis was performed in the Division of Reproductive Medicine (Head Prof.Dr. B.C.J.M. Fauser), Department of Obstetrics and Gynecology, Erasmus Medical Center, Rotterdam, the Netherlands.

The author and studies presented in this thesis were supported by 'Stichting Voortplantingsgeneeskunde Rotterdam' and research grants from Organon International and Wyeth Nederland BV.
Most of this PhD manuscript has been published previously and has been reproduced with permission from the publishers:

van Heusden,A.M. and Fauser,B.C.J.M. (1996) FSH and ovarian function during combined oral contraceptive regimens. In Fauser,B.C.J.M. (ed), FSH action and intraovarian regulation. The Parthenon Publishing Group, London, pp. 211-221.

Fauser,B.C.J.M. and van Heusden,A.M. (1997) Manipulation of human ovarian function: physiological concepts and clinical consequences. Endocrine Reviews, 18, 71-106.
van Heusden,A.M. and Fauser,B.C.J.M. (1999) Activity of the pituitary-ovarian axis in the pill-free interval during use of low-dose combined oral contraceptives. Contraception, 59, 237-243.

van Heusden,A.M., Killick,S.R., Coelingh Bennink,H.J. and Fauser,B.C.J.M. (2000) Single monthly administration of the anti-progestagen Org 31710 in users of the 75 microg desogestrel progestagen-only pill: effects on pituitary-ovarian activity. Human Reproduction, 15, 629-636.

van Heusden,A.M., Coelingh Bennink,H.J. and Fauser,B.C.J.M. (2002) FSH and ovarian response: spontaneous recovery of pituitary-ovarian activity during the pill-free period vs. exogenous recombinant FSH during high-dose combined oral contraceptives. Clinical Endocrinology, 56, 509-517.

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Cover design A.M. van Heusden
Typeset, printed and bounded by Optima Grafische Communicatie, Rotterdam

First published 2003

ISBN 90-6734-145-2

Rage, rage against the dying of the light.

Dylan Thomas

Ter nagedachtenis aan mijn moeder,

Aan mijn vader,

Voor Inge en Hugo.

Promotiecommissie

Promotor:	Prof.dr. B.C.J.M. Fauser
Overige leden:	Prof.dr. H.J.T. Coelingh Bennink
	Prof.dr. F.H. de Jong
	Prof.dr. J.W. Wladimiroff

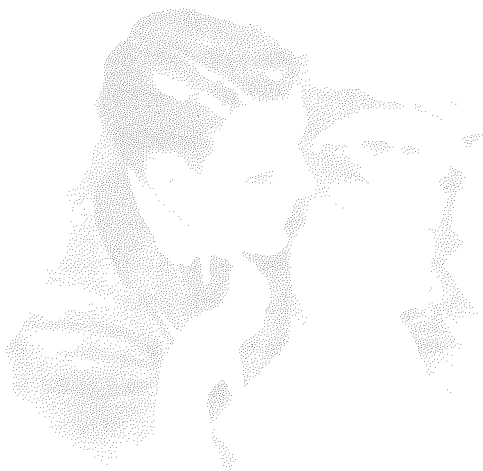
CONTENTS

List of Abbreviations

AUC	area under the curve
BMI	body mass index (weight / height^2)
COC(s)	combined oral contraceptive(s)
DHEAS	dehydroepiandrosterone sulphate
DMPA	depot medroxyprogesterone acetate
DSG	desogestrel
E_2	17β-oestradiol
EE	ethinyl-estradiol
FSH	follicle-stimulating hormone
GnRH	gonadotropin releasing hormone
GSD	gestodene
hCG	human chorionic hormone
hMG	human menopausal gonadotropin
im	intramuscular
IRMA	immunoradiometric assay
iv	intravenous
LNG	levonorgestrel
LH	luteinzing hormone
LYN	lynestrenol)
µg	micrograms
NET	norethisterone
NSG	norgestimate
P	progesterone
uFSH	(purified) urinary follicle-stimulating hormone
POP	progestin-only pill
RIA	radioimmunoassay
sc	subcutaneous
SD	standard deviation
SHBG	sex-hormone binding globulin
T	testosterone
TVS	transvaginal sonography

Chapter 1

Introduction and objectives



1.1 General Introduction

The work of the Austrian Ludwig Haberlandt in the 1920's with regard to the administration of ovarian and placental extracts to animals, led to the concept of oral contraception (Haberlandt, 1921). This occurred prior to the any knowledge of steroid hormones. The isolation of progesterone by Corner and Allen in 1933, the synthesis of progesterone by Butenandt and Schmidt in 1934 and, finally, the synthesis of progesterone from plant (Mexican yam) derived diosgenin by Marker in 1940 led to commercial synthesis of progesterone by Syntex in 1944. The synthesis of norethisterone (Djerassi, Syntex) in 1951 and norethynodrel (Colton, Searle) in 1952 heralded the use of orally active progestins (Perone, 1994).

In the 1950's, Pincus, Rock and Chang started their studies (initialized by Margaret Sanger and Katherine McCormick who provided a research fund) to develop a safe and effective contraceptive. Ironically, it was from the perspective of pro-fertility that motivated Rock to treat women with progestins. Once the prevention of ovulation was evident during progestin use, this led to a comparative trial using 3 synthetic progestins. One of them, norethynodrel proved to be contaminated with the estrogen mestranol. Once mestranol was successfully removed from the medication, inhibition of ovulation proved to be less consistent and break-through bleeding was observed more frequently. This provided the basis for combining progestins with an estrogen in the formulation of the Pill. Finally the start of larger studies in Puerto Rico in 1958 led to data that allowed Searle to get the first oral contraceptive (Enovid-10 ®) approved by the FDA. This first combined oral contraceptive (COC) combined 150 mcg mestranol and 9.85 mg norethynodrel (Asbell, 1995; Pincus, 1956; Pincus *et al.*, 1958; Pincus *et al.*, 1959).

Oral contraceptives inhibit ovarian activity and ovulation through negative feedback actions of administered synthetic steroids on the hypothalamic-pituitary axis (Figure 1). The oestrogen compound is believed to primarily inhibit FSH secretion, whereas progestins are supposed to mainly inhibit LH. Key effects of progestins involve reducing the frequency of the hypothalamic GnRH pulse generator. However, the contention of disparate effects of steroids on gonadotropin release has not been carefully investigated, and studies comparing the effects of estrogens alone vs. estrogen/progestin combinations on pituitary-ovarian function are lacking.

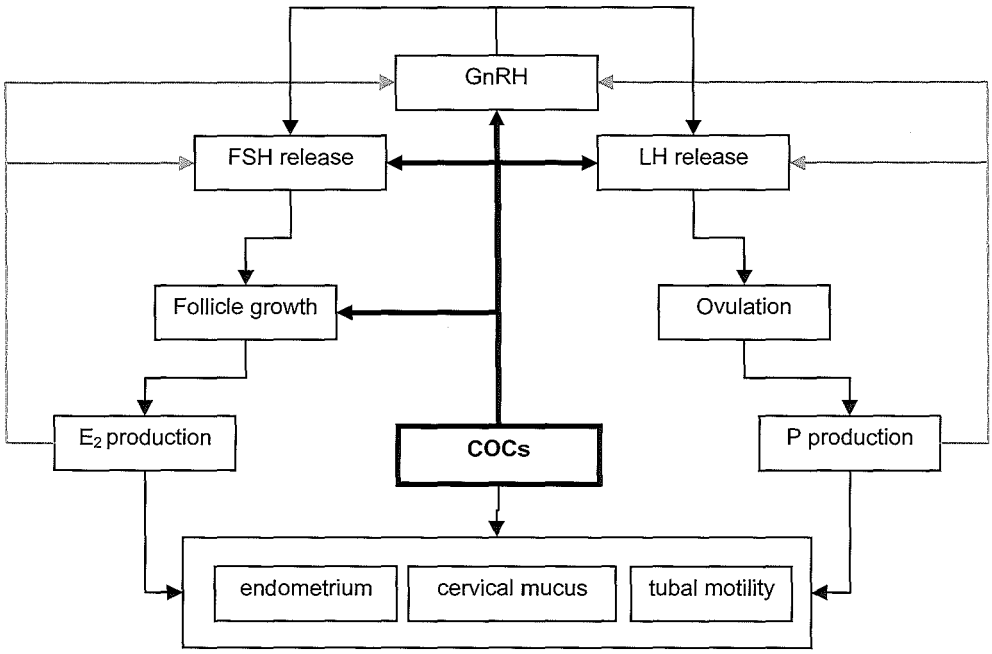


Figure 1 Proposed inhibitory pathways of oral contraceptives

Steroid contraception is well tolerated, exceptionally effective, and extensively used worldwide. In an attempt to reduce side effects and to diminish the potential for short and long-term complications, estrogen doses have been gradually reduced. Since the introduction of oral contraceptives in the early 1960s, daily doses of ethinylestradiol (EE) in commercially available preparations have been diminished from 150 to 20 mcg (Kaunitz, 1998; Poindexter, 2001; Teichmann *et al.*, 1995). Combined steroid pills with EE doses as low as 10 mcg/day have proven effective when medication is taken correctly (Preston, 1974). Combined steroid contraceptives containing 1 mg of micronized estradiol have also been shown to inhibit ovulation, although control of bleeding was insufficient (Wenzl *et al.*, 1993). In addition, novel, so-called second and third generation, progestins with reduced androgenic side effects have been developed and introduced in contraceptive regimens (Henzl, 1993). Progestins may be combined with estrogens or may be administered alone. Progestin only (oral and depot) preparations have been tested extensively in recent years to provide women with the alternative of estrogen-free contraceptives. However, reduced suppression of pituitary FSH release introduced the need for continued progestin medication, which negatively affects cycle control (McCann and Potter, 1994).

Although pill effectiveness has not been compromised substantially, diminished suppression of circulating FSH by reduced steroid doses may give rise to substantial residual ovarian activity, as well as reduced tolerance for pill omission or for other circumstances that reduce circulating steroid concentrations. Follicle growth and concomitant E_2 production usually occur during the pill-free interval and the first week of pill intake, or when tablets are missed. Pill omission has been reported to occur in a substantial proportion of pill users in everyday practice (up to 47% of women in a 3-month period) (Finlay and Scott, 1986b; Klitsch, 1991; Rosenberg and Waugh, 1999) and is clearly associated with contraceptive failure (Fraser and Jansen, 1983; Grady *et al.*, 1986;Trussell and Kost, 1987) In the great majority of studies published so far, monitoring of ovarian function is performed infrequently (screening intervals usually varies between twice weekly or once every month), and hormone assays and ultrasound for the assessment of follicle growth are rarely combined. However, substantial ovarian activity is uniformly reported when women use steroid regimens that are presently on the market. The concept arises that FSH levels rise during the pill-free interval above the 'threshold' for follicle recruitment, and that follicle growth around the stage of dominant follicle selection is usually arrested after initiation of the next pill cycle (Van Heusden and Fauser, 1996). Improved understanding of ovarian activity during oral contraceptive medication may help to design novel strategies for steroid contraception.

1.2 Study objectives

The study objectives in this thesis focus on pituitary-ovarian activity in women using oral contraceptive steroids. Contraceptive steroids influence the hypothalamic-pituitary-ovarian axis in order to interfere with normal follicular development and ovulation. Additional effects on the endometrium and cervical mucus may also attribute to the contraceptive effects established by contraceptive steroids. Despite the described additional effects on which the ultimate contraceptive efficacy is based, inhibition of ovulation provides a most reliable form of contraception. The first objective was to review the current knowledge on residual ovarian activity in users of oral contraceptive steroids (see chapter 2). The first study (described in chapter 3) was performed to evaluate to relative importance of dosage of ethinyl-estradiol and type of progestin regarding pituitary-ovarian activity during the pill-free period in users of low-dose combined oral contraceptives. The second study was performed to compare recovery of the pituitary-ovarian activity during the pill-free interval with the ovarian response obtained from different dosages of recombinant FSH during high-dose combined oral contraceptives (chapter

4). The third and fourth study were performed to evaluate the effect of a single dose of an antiprogestin during continuous progestin-only contraception: Both the effects on the pituitary-ovarian axis (chapter 5) and the effects on endometrial characteristics and bleeding patterns (chapter 6) are described.

Conclusions derived from the studies described in this thesis are presented in the general discussion.

Chapter 2

Residual ovarian activity during oral steroid contraception

2.1 Introduction

Oral steroid contraceptives have emerged as a generally well tolerated, reversible, effective form of contraception and are extensively used worldwide. Owing to the pioneer work of Pincus et al (Pincus *et al.*, 1958), the first combined oral contraceptive (COC) containing 150 mcg mestranol and 9.85 mg norethynodrel was developed in the late 1950's. Since the introduction of this first COC, new contraceptive techniques provide alternative forms of steroid contraception: intramuscular depot injections, subdermal implants, intra uterine delivery systems, vaginal rings and transdermal patches. Oral contraceptives evolved through discovery of new progestins, development of progestin-only pills and the gradual lowering of the oestrogen content in COCs. Bi-phasic and tri-phasic regimens appeared by changing the individual dosages for the oestrogen and progestin components. All these efforts are aimed at reducing adverse effects and improving compliance while maintaining superior efficacy over non-steroid forms of reversible contraception. However, in reducing the steroid burden to avoid adverse effects, suppression of endogenous hypothalamic-pituitary-ovarian activity could be compromised in case of pill omissions, during the pill free period and during co-medication (Fauser and Van Heusden, 1997). Studies addressing the influence of contraceptive steroids on the hypothalamic-pituitary-ovarian axis can be classified as shown in Table 1.

The exact mode of action of contraceptive steroids in COC has not yet been satisfactorily elucidated. The majority of studies describe serum levels of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), oestradiol (E_2) or progesterone (P) sometimes in combination with ultrasound assessments of follicle diameters during the studied contraceptive medication. Next to these observational studies knowledge concerning contraceptive activity of COCs has benefited from intervention studies. Gonadotrophin-releasing hormone (GnRH), human chorionic gonadotrophin (hCG) or FSH intervention studies during active medication, pill-omissions and the study of the pill-free period have provided critical information regarding contraceptive efficacy. The focus of this review is to summarize the effects of oral steroid contraceptives on residual pituitary-ovarian activity.

Table 1

Differences in features of studies that address the influence of contraceptive steroids on the pituitary-ovarian axis.

Study medication

oral oestrogen/progestagen users (mono-, bi- and triphasic schedules)

oral progestagen-only users (mini-pill)

systemic high-dose progestagen only users (e.g. DMPA)

systemic oestrogen/progestagen users (long-acting injectables, medicated vaginal rings)

systemic low-dose progestagen only users (e.g. Norplant® , Implanon®)

Methods

Serum FSH, LH, E_2 , progesterone (daily, weekly, monthly, other condition)

Ultrasound assessments of follicle diameter (daily, weekly, monthly, other condition)

Setting

Normal use (mode of action, comparative trials)

Discontinuation of use (and subsequent return to 'fertility')

Intervention (e.g. GnRH administration, hCG or FSH injections, pill omissions)

Outcome

Classification of ovarian activity

Ovulations (either based on progesterone level, ultrasound features or both)

Dominant follicles (follicle diameters > 10 mm)

Descriptive data on hormones

Descriptive data on follicle growth

(Pregnancy)

Pitfalls

Different hormone assays FSH, LH, E_2 and P

Sub-optimal timing/frequency study parameters

Different criteria for interpretation of the study data

Biased study population

Dissimilar effects of studied progestins

Large intra- and inter-individual variations in pharmacokinetics of used drug(s)

Large intra- and inter-individual variations hypothalamic-pituitary-ovarian response

DMPA = depot medroxyprogesterone acetate

2.2 Progestins

The classification of progestins is based either on chemical structure (19-nortestosterone derivatives or 17-α-acetoxyprogesterone derivatives), receptor affinity or 'relative' potency. The latter is often derived from their effects on the endometrium (Clauberg test or Swyer-Greenblatt test) (Goldzieher, 1986). However, equipotency in relation to endometrial effects does not imply the same effect on other parameters such as metabolism or LH suppression. Thus the assessed 'relative potency' depends upon the target organ and the parameter studied and therefore should not be used.

Progestins are believed to exert their contraceptive effects through a variety of changes in different targets. (1) Suppression of the LH surge and ovulation (Tafurt *et al.*, 1980; Barnhart *et al.*, 1997; Van Heusden *et al.*, 2000). (2) Changes in permeability of cervical mucus (Kumar *et al.*, 1991; Croxatto *et al.*, 1987). (3) Changes in endometrial receptivity for embryo implantation (Landgren *et al.*, 1990; Song and Fraser, 1995; Somkuti *et al.*, 1996). (4) Changes in tubal and uterine motility causing delayed gamete transport (Coutinho *et al.*, 1973; Paltieli *et al.*, 2000) and (5) a direct effect on the ovary (Kim Bjorklund *et al.*, 1992; Dericks-Tan *et al.*, 1992; Kuhl, 1996). The contraceptive effects of progestins on endometrium and tubal motility are speculative. However, in high doses used for postcoital contraception they appear to induce a potent contraceptive effect (Wu *et al.*, 1999; Ling *et al.*, 1983; Hapangama *et al.*, 2001).

A major target of progestins is to inhibit the LH surge in order to prevent ovulation. During the normal menstrual cycle there is a noticeable variation in the spontaneous pulsatile release of LH and response to a GnRH bolus (Yen *et al.*, 1972; Wang *et al.*, 1976). Progesterone induces low amplitude GnRH pulses and is able to block the E_2 induced LH surge at the hypothalamic level (Wildt *et al.*, 1981b; Hanker *et al.*, 1985). Administration of progestins probably induces changes in the pulsatile release of LH comparable with the late luteal phase, although this is predominantly demonstrated in women using COCs. Two studies in progestin-only users - long-term injectables depot medroxyprogesterone acetate (DMPA) (Perez-Lopez *et al.*, 1975) or norethindrone-enanthate (NET-EN) (Ismail *et al.*, 1987) - fail to demonstrate differences in basal or GnRH induced gonadotrophins compared to controls. This suggests that the pituitary is not a primary site for ovulation inhibition by these progestins. No data are available with regard to the effects of oral progestins on GnRH-stimulated pituitary response.

Although progestin-induced changes in the hypothalamic-pituitary signalling lack definite proof, changes in pituitary-ovarian activity are well documented. Studies performed by Landgren and co-workers (Landgren *et al.*, 1979; Landgren and Diczfalusy, 1980; Landgren *et*

al., 1981) regarding the effects of 300 mcg norethisterone revealed 4 types of ovarian activity: (1) no activity, although FSH levels were not suppressed (16%), (2) follicle growth without luteal activity along with suppressed LH concentrations(23%), (3) normal follicular development and inadequate luteinization (21%) and (4) normal ovulatory cycles (40%). FSH and LH peak values were suppressed but mean levels remained normal. No difference in LH levels was found among women showing different types of ovarian response. In these studies, E_2 and P levels comparable to an ovulatory cycle were seen despite lowered or even absent LH and FSH surge. Thus, ovarian suppression by 300 mcg norethisterone alone was unrelated to the degree of inhibition of FSH and LH secretion suggesting direct interference with ovarian function. Other studies confirm an unpredictable effect of norethisterone on ovulation suppression (Nuttall *et al.*, 1982; Song *et al.*, 1993) also when given intranasally (Shah *et al.*, 1985; Anand Kumar *et al.*, 1991). Tayob confirmed ovulation by ultrasound (Tayob *et al.*, 1985; Tayob *et al.*, 1986) and discovered a higher incidence of functional ovarian cysts in progestin-only pill users. In women using 75 mcg desogestrel daily, however, complete suppression of ovulation was established while dominant follicles and moderate E_2 levels commonly were present (Rice *et al.*, 1996; Van Heusden *et al.*, 2000). Data obtained from progestin implants (Norplant®, Implanon®, Uniplant®) indicate a variable degree of ovarian activity ranging from normal ovulatory cycles, luteal phase defects, luteinized unruptured follicles and complete suppression of follicle development (Brache *et al.*, 1985; Shaaban *et al.*, 1984; Shaaban *et al.*, 1993; Alvarez *et al.*, 1986; Alvarez *et al.*, 1996; Croxatto *et al.*, 1988; Faundes *et al.*, 1991; Makarainen *et al.*, 1998; Shoupe *et al.*, 1991; Brache *et al.*, 2000; Devoto *et al.*, 1997; Barnhart *et al.*, 1997). The high degree of ovulation inhibition achieved with progestin-only implants seems to place this strategy between oral progestin-only and injectable progestin-only contraceptive methods pertaining to contraceptive efficacy.

Summarizing the effects of oral progestins on the hypothalamic-pituitary ovarian axis it seems clear that many issues remain unsolved. The direct effect of synthetic progestins on hypothalamic GnRH secretion remains poorly understood. Pituitary function is erratic and results in a wide variety of ovarian activity including ovulation. The suppression of gonadotrophins (especially LH) appears to be dependent on both dose and type of progestin. Direct effects of progestins on the ovary are suggested but remain to be established. While some progestins can achieve high contraceptive efficacy when ovulation suppression is almost complete, undesirable bleeding-patterns seem to prevent its widespread use.

2.3 Antiprogestins

Progesterone transforms the endometrium from a proliferative to a secretory state in the normal menstrual cycle. During the late follicular phase progesterone also facilitates the LH surge, which induces ovulation (Lasley *et al.*, 1975; Hoff *et al.*, 1983). Consequently, antiprogestins may also have contraceptive potential (Swahn *et al.*, 1996). Current knowledge on antiprogestins is derived from human and non-human studies (primates and rodents) in which different antiprogestins are used. Additional studies are needed to corroborate the data derived from animal studies and to identify disparity in the effects of different antiprogestins.

Mifepristone (RU 486), the prime example of a progesterone antagonist, is a 19-norsteroid with anti-progesterone and anti-glucocorticoid properties (Bygdeman *et al.*, 1993). However, in certain circumstances, antiprogestins may act as progesterone agonists or anti-oestrogens. Its primary use is in post-coital contraception and termination of early pregnancy (Spitz *et al.*, 1996; Spitz *et al.*, 2000; Task Force on Postovulatory Methods of Fertility Regulation, 1999; Bygdeman *et al.*, 2000). Anti-progestins are able to establish contraceptive effects through delay of folliculogenesis, ovulation inhibition and interference with normal endometrial development. These effects depend on dosage and phase of the menstrual cycle.

During the follicular phase, administration of antiprogestins interferes with follicle growth and ovulation in a dose dependent fashion. Single doses of antiprogestins administered before the LH peak, delay the LH surge, delay folliculogenesis and lengthen the menstrual cycle (Stratton *et al.*, 2000; Collins and Hodgen, 1986; Baird *et al.*, 1995). Single or repeated administration arrests follicle development, inhibits a rise in E_2 and decreases plasma inhibin concentrations, even in low dosages (Croxatto *et al.*, 1995; Brown *et al.*, 2002). However, in case the delay is followed by ovulation, it does not affect implantation (Ghosh *et al.*, 1997). Although it is generally assumed that the principle mode of action is established through inhibition of E_2 feedback, inhibitory effects at the hypothalamus (Heikinheimo *et al.*, 1996; Heikinheimo *et al.*, 1995; Kazem *et al.*, 1996), the pituitary (Sanchez-Criado *et al.*, 1999; Van Uem *et al.*, 1989; Wolf *et al.*, 1989a) or a direct effect at the ovary (Messinis *et al.*, 1997; Dimattina *et al.*, 1986) have also been suggested.

Antiprogestins administered as a single high dose following the LH peak will delay development of secretory changes in the endometrium without affecting the length of the luteal phase (Cameron *et al.*, 1997; Gemzell-Danielsson *et al.*, 1994; Kohler *et al.*, 1984). This is achieved through the inhibition of progesterone-dependent down regulation of oestrogen and progesterone receptors in the endometrium (Cameron *et al.*, 1996). Late luteal administration of

a single high dose does not achieve reliable contraceptive effects (Couzinet *et al.*, 1990). Very low daily doses of antiprogestins induce discrete changes in the endometrium (Danielsson *et al.*, 1997) resulting in reduced pregnancy rates (Marions *et al.*, 1999; Zelinski-Wooten *et al.*, 1998). Low-dose administration once a week does not inhibit ovulation, but delays endometrial development and impairs secretory activity (Gemzell-Danielsson *et al.*, 1996). Thus a significant decrease in pregnancy rate could be established without affecting the menstrual cycle (Marions *et al.*, 1998) even when ovulation is not consistently inhibited (Croxatto *et al.*, 1998; Katkam *et al.*, 1995; Spitz *et al.*, 1993).

Although continuous anti-progestin administration can prevent ovulation, it also affects cycle length and allows for continuous (unopposed) influence of oestrogen on the endometrium. Primate studies on the antiprogestin ZK 137 316 indicate that inhibition of progesterone action together with a blockade of oestrogen-dependent proliferation can result in endometrial atrophy (Slayden *et al.*, 1998). Combining progestins and anti-progestins could allow for regular cycles while maintaining anovulation. However, simultaneous and sequential cyclic co-administration of a progestin with an anti-progestin abolishes the antiovulatory action of the antiprogestin or the progestin (Kekkonen and Lahteenmaki, 1996; Croxatto *et al.*, 1989; Kekkonen *et al.*, 1995; Van Heusden *et al.*, 2000) but generally improves bleeding patterns.

In conclusion, the most reliably form of contraception using antiprogestins is established when given as high single dose during the mid-cyclic phase, rendering it a rather unpractical approach. Daily doses either block ovulation but may cause an unopposed oestrogen effect on the endometrium, depending on the type of antiprogestin used. Low doses which do not affect ovulation, delay endometrial maturation of which the contraceptive effect lacks convincing proof.

2.4 Oestrogens

It was Greenblatt who demonstrated that conjugated equine oestrogens or pellets of crystalline E_2 could inhibit ovulation (Greenblatt and Zarate, 1967; Emperaire and Greenblatt, 1969; Greenblatt *et al.*, 1974; Greenblatt *et al.*, 1977). A subsequent study established the effect of different dosages of oestrogen alone or in combination with a progestin on pituitary gonadotrophin release. A progressive dose-dependent suppression of LH and FSH was observed while the addition of a progestin dramatically increased the magnitude of gonadotrophin inhibition (Goldzieher *et al.*, 1975). Later, subcutaneous E_2 implants (Magos *et al.*, 1987) and E_2 transdermal patches (Watson *et al.*, 1988) were also shown to be able to suppress ovulation. The exact mode of action by which oestrogens induce suppression of ovulation remains to be elucidated. In castrated postmenopausal women, oestrogen implants suppress gonadotrophin levels suggesting a direct effect at the pituitary and/or hypothalamus (Thom *et al.*, 1981). Inhibitory and stimulatory effects of oestrogens on gonadotrophin secretion (Yen and Tsai, 1971) along with differential effects on LH and FSH have been reported. Studies in primates suggest that the primary site of suppression of gonadotrophins is at the pituitary (Knobil, 1980). Also in primates, oestrogens were able to inhibit growth of preantral and medium-sized antral follicles presumably through a direct effect at the ovary (Koering *et al.*, 1991; Koering *et al.*, 1994).

2.5 Combined Oral Contraceptives

Traditionally, the doctrine with regard to the mode of action of steroidal components in COC implicated progestins to inhibit the LH surge whereas oestrogens are required for endometrial stability and thus providing satisfactory bleeding patterns. This concept is understandable from a historical perspective, since oestrogens were found fortuitously as an impurity in the first contraceptive medication. Currently a wide variety of changes attributed to both steroid components should contribute to the contraceptive effects of COCs. Decreased gonadotrophin secretion, inhibition of the LH surge, altered responsiveness to gonadotrophin stimulation, decreased cervical mucus permeability, decreased endometrial receptivity for implantation, decreased motility of the oviduct and uterus impairing gamete transport, or a direct suppressive effect on the ovary have all been proposed to explain the contraceptive efficacy of steroids.

A large amount of descriptive data is available with regard to the effect of COCs on gonadotrophin and ovarian steroid levels as well as ultrasound assessments of follicular dynamics (see Tables 2-5 for detailed description of relevant studies). Various investigations have been performed describing parameters of pituitary-ovarian activity during oral contraceptive regimens: alternative start of a medication-strip (Sunday-starters), effect of pill-omission at different moments during the pill strip, regular and extended pill-free periods and comparing different formulations during normal use.

2.5.1 Normal use

The general conclusion from studies performed to investigate pituitary-ovarian activity during COC use (for overview of individual studies see Table 2), is a gradual decline of gonadotrophins in the first week of medication leading to suppression of development of non-dominant follicles and subsequent E_2 demise. In case dominant follicles are present at the start of COC, they can still increase in diameter and even reach cystic proportions (> 30 mm). Delayed start of a new COC cycle (i.e. 'Sunday starters') increases the risk of dominant follicles to emerge and should therefore be discouraged (Killick *et al.*, 1987; Danforth and Hodgen, 1989). Despite increase in follicular diameter during active medication, E_2 levels decrease eventually probably as a result of very low LH levels due to the two-gonadotrophin two-cell hypothesis (Schoot *et al.*, 1992). Although this raises questions with regard to 'the functional life span' of these follicles (Faundes *et al.*, 1996; Shaw *et al.*, 1992; Kettel *et al.*, 1991), it appears that the potential to ovulate remains (Killick, 1989).

Virtual all commercially available COCs combine ethinyl-estradiol (some of the older preparations in the US still contain mestranol) with a synthetic progestin. A new combination containing 1 mg micronized 17-β-oestradiol (1 mg E_2 + 150 mcg desogestrel) has been shown to inhibit ovulation (Csemiczky *et al.*, 1996; Schubert and Cullberg, 1987; Wenzl *et al.*, 1993). The additional contraceptive effect of E_2 remains to be established since 75 mcg desogestrel alone also appears to be able to inhibit ovulation effectively (Van Heusden *et al.*, 2000). The extent of pituitary-ovarian suppression appears to be related to the dose of ethinyl-estradiol in COCs while the type of progestin is less important (Van Heusden and Fauser, 1999; Mall Haefeli *et al.*, 1991; Spellacy *et al.*, 1980; Fauser and Van Heusden, 1997).

Table 2

Summary of studies on pituitary-ovarian activity during combined oral contraception.

I: normal use

Reference	Medication *(number of subjects)*	Ultrasound assessments *(follicles > 10 mm)**			Endocrine assessments
Elstein 1974	50 mestranol + 1000 NET (n=5)				no ovulation
Kuhl 1985	30 EE + 50/75/125 LNG (n=11) 30 EE + 150 DSG (n=11)				3 ovulations .
Doyen 1987	several low-dose OC's; (n=35)		12%		no ovulation
Killick 1987	30/40/30 EE + 50/75/125 GSD (n=22)		36%		No LH surges, no elevations of P
vd Vange 1988	7 low dose oral contraceptives (n=70) cycle *1, 3 & 6*		*>10mm*	*> 18 mm*	6 suspected ovulations
		1	29%	27%	
		3	29%	29%	
		6	30%	31%	
Jung-Hoffman 1988	1 30 EE + 75 GSD (n=11) 2 30 EE + 150 DSG (n = 11)				1 ovulation
Westcombe 1988	30/40/30 EE + 50/75/125 LNG 1 switchers (n=25) 2 first-users (n=21) started on CD 5				Ovulation switchers: 4% first-users: 24%
Hamilton 1989	35 EE + 500/750/1000 mg NET (n=30)		37%		
Thomas 1990	30 EE + 75 GSD (n=25) *pre*-study cycle, cycle *1, 2 & 3* and *post*-study cycle	*pre*	100%		no luteal activity during OC use
		1	33%		
		2	22%		
		3	44%		
		post	100%		
Young 1992	Sunday starters *1* 20/30/35 EE + 1500 NET (n= 16) *2* 30 EE + 1500 NET (n=16) *3* placebo (n=16)			*> 18 mm*	1 pregnancy in group 2
		1		43%	
		2		25 %	
		3		63%	

Shaw	30/40/30 EE + 50/70/100 GSD (n=25)	*1*	28%			No ovulations
1992	*cycle 1, 2, 6*	*2*	36%			
		6	44%			
Grimes	Sunday starters			*> 30 mm*		ovulation
1994	*1* 35 EE + 500/750/1000 NET (n=10)					
	2 35 EE + 1000 NET (n=11)	*1*	52%	10%		1 2%
	3 35 EE + 500 NET (n=11)	*2*	29%	5%		2 0%
	4 controls (n=10)	*3*	50%	1%		3 5%
		4	62%	7%		4 70%
Fitzgerald	1 20 EE + 75 GSD (n=27)	*cycle*	*1*	*2*	*3*	No ovulations,
1994	2 20 EE + 150 DSG (n=26)					1 LUF cycle in DSG
		DSG	31%	37%	33%	group
	3 cycles	GSD	16%	43%	27%	
Broome	*1* 30/40/30 EE + 50/75/125 LNG (n= 17)		*<10mm*	*10 - 30*	*> 30 mm*	
1995	*2* 30 LNG only or 350 NET only (n=15)					
	3 control (n=10)	*1*	24%		18%	
		2	67%	53%		
		3	70%	10%		
vd Does	*1* 30/40/30 EE + 50/75/125 LNG (n=15)			*> 15mm*		1 LUF syndrome in
1995	*2* 35/30/30 EE + 50/100/150 DSG (n=16)					group 1
		1	63%	44%		1 ovulation in group 2
		2	73%	60%		
Teichmann	*1* 30 EE + 75 GSD (n=153)			*10-30mm*	*> 30 mm*	no ovulations observed;
1995	*2* 20 EE + 150 DSG (n= 149)					1 pregnancy in group 2
		1		18%	5%	
		2		10%	2%	
Crosignani	*1* 35/30/30 EE + 50/100/150 DSG (n=22)					No ovulations
1996	*2* 20 EE + 150 DSG (n=15)	*1*	11%			
	3 20 EE + 75 GSD (n=14)	*2*	13%			
		3	7%			
Spona	20 EE + 100 LNG (n=24)		*>10*	*>13mm*		1 women (2 cycles)
1996	*3* cycles					LUF
		1	17%	8%		
		2	13%	46%		
		3	9%	33%		

Rabe 1997			*day 10-12*	*day 16-18*	No ovulations
	1 controls (n=108)	*1*	44%	34%	
	2 20/50 EE/LNG (n=83)	*2*	6%	9%	
	3 30/40/30 EE + 50/75/125 LNG (n=53)	*3*	25%	9%	
	4 20 EE + 150 DSG (n=47)	*4*	2%	2%	
	5 30 EE + 150 DSG (n=59)	*5*	3%	7%	
	6 30 EE + 75 GSD (n=16)	*6*	17%	8%	
	7 35 EE + 250 NGS (n=33)	*7*	13%	16%	
	8 35/35/35 EE + 180/215/250 NGS(n=38)	*8*	17%	15%	
Coney 1999	20 EE + 100 LNG (n=26)			3% LUF 3% ovulation	

All medications are abbreviated (e.g. 30 EE + 150 DSG means 30 mcg ethinyl-estradiol combined with 150 mcg desogestrel) : EE (ethinyl-estradiol) NET (norethisterone), LNG (levonorgestrel), DSG (desogestrel), GSD (gestodene), NSG (norgestimate), LYN (lynestrenol)

* dominant follicles (Pache *et al.*, 1990)

2.5.2 *Pill-omissions*

The effect of pill omissions during COC use is related to the moment in the pill cycle and the number of pills missed (for summaries of relevant studies see Table 4). Suppression of pituitary-ovarian activity increases with the number of pills already taken and maximum suppression is often encountered at the end of the COC cycle (Smith *et al.*, 1986). High-dosed COCs may reach maximum suppression even after 7 days (Van Heusden *et al.*, 2002). Once maximum suppression is achieved, up to 7 pills can be omitted (i.e. the pill free period). Extending these 7 days easily leads to the development of dominant follicles that continue to grow, even when OC therapy is recommenced (See also Figure 1). The administration of hCG was able to induce ovulation and luteinization in these women (Killick, 1989). However, the initial LH peak after stopping COC occurred 21 to 28 days thereafter (Klein and Mishell, 1977). This effect may be ethinyl-oestradiol dose related (Bracken *et al.*, 1990). Determination of the moment of maximum suppression of pituitary-ovarian activity during any COC regimen is therefore mandatory to allow safe instructions for women who omit pills (Molloy *et al.*, 1985; Guillebaud, 1987). The high prevalence of pill-omissions (up to 27 % in 3 months) (Finlay and Scott, 1986a; Klitsch, 1991) and the subsequent risk of contraceptive failure (Fraser and Jansen, 1983; Grady *et al.*, 1986; Trussell and Kost, 1987) indicate the necessity to increase contraceptive safety to cope with the

problem of pill omissions.

Table 3

Summary of studies on pituitary-ovarian activity during combined oral contraception.

II: pill omissions

Reference	Medication *(number of subjects)*	Ultrasound assessments (follicles > 10 mm) *	Endocrine assessments
Chowdhury 1980	30 EE + 1000 NET (n=54) 2 consecutive missing pills day 7-17 1 1st cycle (n=35) 2 4th cycle (n=19)		Ovulations 1/10 in control group 10/35 in first cycle 7/19 in fourth cycle
Wang 1982	30 EE + 150 LNG (n=32) 1 omission day 9 and 10 (n=8); 2 11 and 12 (n=8); 3 14 and 15 (n=8); 4 17 and 18 (n=8)		No ovulations
Smith 1986	1 30 EE + 150 LNG (n=18) 2 30/40/30 EE + 50/75/125 LNG (n=18) 7, 14 or 21 days of study medication		1 ovulation following single week of triphasic medication
Letterie 1992	35 EE + 500/750/1000 NET (n=15) 1 1 missing tablets day 1-4 (n=5) 2 2 missing tablets day 3-6 (n=5) 3 3 missing tablets day 6-9 (n=5)	no follicles > 13mm	No ovulations
Hedon 1992	35 EE + 250 NGS (n=47) control cycles (n=5) 2 cycles 1-4 pill omissions on day 1, 6, 12, 18	19 % follicles > 10 mm	No ovulations
Vallon 1992	35 EE + 250 NGS (n = 39) 2 study cycles 1-4 pill-omissions on day 1, 6, 12 or 18; 5 controls	follicle > 16 mm : 6/39	No ovulations

All medications are abbreviated (e.g. 30 EE + 150 DSG means 30 mcg ethinyl-estradiol combined with 150 mcg desogestrel) : EE (ethinyl-estradiol) NET (norethisterone), LNG (levonorgestrel), DSG (desogestrel), GSD (gestodene), NSG (norgestimate), LYN (lynestrenol)

2.5.3 *The pill-free period*

The standard regimen of COCs allows for a pill-free (or at least steroid-free) interval of generally 7 days for eliciting regular withdrawal bleedings. During this period, pituitary-ovarian activity recovers following the waning inhibitory effects of contraceptive steroids. Consequently, gonadotrophin-dependent follicle growth is initiated until the beginning of the next medication strip after which inhibition of the pituitary-ovarian axis is re-established. Events during the pill-free period resemble those observed during the early follicular phase of the normal menstrual cycle (Figure 1). FSH levels increase above the threshold for ovarian stimulation allowing gonadotrophin-dependent follicle growth and subsequent oestrogen production (Fauser and Van Heusden, 1997; Macklon and Fauser, 2000). Single dominant follicle development in the normal menstrual cycle coincides with a decrease in serum FSH concentrations (Pache *et al.*, 1990; van Santbrink *et al.*, 1995).

Resumption of contraceptive medication at the end of the pill-free period decreases FSH levels irrespective of whether dominant follicles are present or not. If no dominant follicles are present, complete suppression of folliculogenesis ensues providing optimal contraceptive safety. Should dominant follicle selection have occurred during the pill-free period, follicle growth is likely to continue during OC treatment (Van Heusden and Fauser, 1999; Van Heusden *et al.*, 2002). Under these circumstances contraceptive efficacy depends on prevention of ovulation achieved through LH-surge inhibition. The decrease in the oestrogen content of low-dose COCs therefore increases the risk of the development of dominant follicles during the pill-free period. Either reduced pituitary-ovarian suppression at the beginning of the pill-free period or less suppression at the beginning of a new OC cycle allows for an increased duration of the FSH window resulting in dominant follicle development (see Figure 1). Consequently, a further reduction of the oestrogen dose in COC should be accompanied by a change in the paradigm of a 7 day steroid-free interval (Van Heusden and Fauser, 1996).

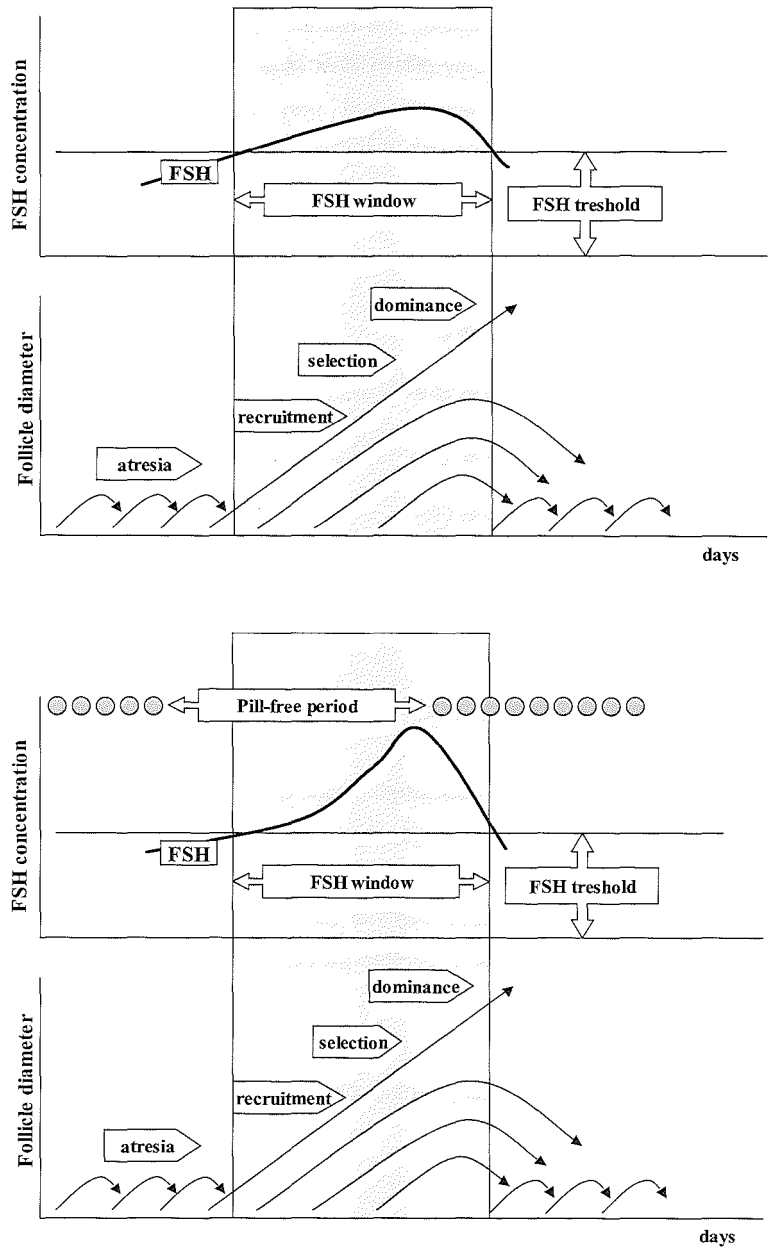


Figure 1 Schematic representation of changes over time in serum FSH concentrations and ovarian follicles comparing the luteo-follicular transition in normo-ovulatory women (upper panel) and the pill-free period in COC users (bottom panel).

Shortening of the pill-free period by increasing the number of contraceptive pills per cycle (Spona *et al.*, 1996a) or adding oestrogen-only pills (Killick *et al.*, 1998) more effectively suppresses the recovery of pituitary-ovarian activity and may therefore increase the contraceptive efficacy of sub-30 COCs. Some strategies to reduce the pituitary-ovarian activity during the pill-free period are summarized in Figure 2.

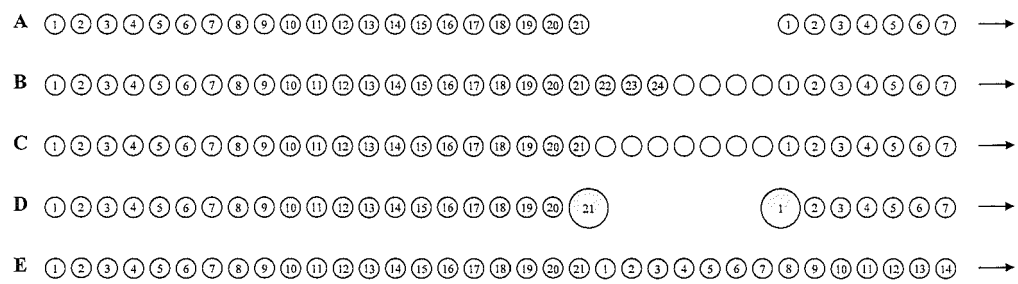


Figure 2 Strategies for reducing residual pituitary-ovarian activity during the pill-free period in combined oral contraceptives.

A : traditional pill-free period of 7 days between 2 medication cycles of 21 days

B : shorten the pill-free period e.g. 24 active pills and 4 placebo

C : use progestin-only or oestrogen-only medication during former pill-free period

D: start and finish 21 medication cycle with high-dose 'sentinel' pill

E : continuous OC use until bleeding occurs and allow pill-free period of 7 days

Table 4 and 5 provide a summary of studies concerning pituitary-ovarian activity during a normal and an extended pill-free interval.

Table 4

Summary of studies on pituitary-ovarian activity during combined oral contraception.

III: normal pill-free interval

Reference	Medication *(number of subjects)*	Ultrasound assessments (follicles > 10 mm) *	Endocrine assessments
Tayob 1990	several low-dose COC's (n=120)	23%	
Elomaa 1998	*1* 30 EE + 75 GSD (n=34)	>12 mm	No ovulations
	2 30/40/30 EE + 50/70/100 GSD (n=34)	*1* 9%	
	3 20 EE + 150 DSG (n=31)	*2* 6%	
		3 27%	
van Heusden 1999	*1* 20 EE + 75 GSD (n=15)	*1* 27%	No ovulations
	2 20 EE + 150 DSG (n=17)	*2* 18%	
	3 30 EE + 150 DSG (n=12)	*3* 0%	
Sullivan 1999	15 EE + 60 GSD		1 ovulation and 6 LUF in 7day PFP group
	1 7 day PFP (n=24)	*1* 64%	
	2 4 day PFP (n=27)	*2* 75%	

All medications are abbreviated (e.g. 30 EE + 150 DSG means 30 mcg ethinyl-estradiol combined with 150 mcg desogestrel) : EE (ethinyl-estradiol) NET (norethisterone), LNG (levonorgestrel), DSG (desogestrel), GSD (gestodene), NSG (norgestimate), LYN (lynestrenol)

Table 5

Summary of studies on pituitary-ovarian activity during combined oral contraception.

IV: extended pill-free interval

Reference	Medication *(number of subjects)*	Ultrasound assessments (follicles > 10 mm) *			Endocrine assessments
Landgren 1984	30 EE + 150 LNG (n= 10) extending pill free interval to 9 days in 2 consecutive cycles				No ovulations
Hamilton 1989	35 EE + 500/750/1000 NET (n=30) 1 pill omission day 1 or 2 cycle 2	7/30 > 14 mm during cycle 2			2 cases of luteinization of which 1 likely to be ovulation
Killick 1989	30/40/30 EE + 50/75/125 LNG (n=10) extending pill-free interval until follicle (12mm) appeared	median time to follicle 12mm after last pill : 11 days (7-16) 2/10 no follicles > 18 mm 8/10 ovulation occurred after 5000IU hCG & follicle > 18mm			
Kilick 1990	1 30 EE + 150 LNG (n=10) 2 30/40/30 + 50/75/125 LNG (n=9) 3 30 EE + 75 GSD (n=9) extended PFP to 9 or 11 days		*> 18 mm* 11%		No ovulations
Landgren 1991	1 30 EE + 150 DSG (n=10) 2 30/40/30 EE + 50 /75/125 LNG (n=10) extended PFP to 10 days				1 ovulation in each group
Elomaa 1998	*1* 30 EE + 75 GSD (n=34) *2* 30/40/30 EE + 50/70/100 GSD (n=34) *3* 20 EE + 150 DSG (n=31) PFP extended to 10 days		*>12 mm* 41% 47% 70%	*> 18mm* 24% 24% 40%	No ovulations 1 LUF in group 1

All medications are abbreviated (e.g. 30 EE + 150 DSG means 30 mcg ethinyl-estradiol combined with 150 mcg desogestrel) : EE (ethinyl-estradiol) NET (norethisterone), LNG (levonorgestrel), DSG (desogestrel), GSD (gestodene), NSG (norgestimate), LYN (lynestrenol)

2.5.4 *Interventions during COC*

Especially in COC users, baseline gonadotrophin levels as well as concentrations following the administration of GnRH are used to assess the magnitude of negative steroid feedback at the hypothalamic-pituitary level. However, the interpretation of these findings is not without difficulties due to the assumption that a diminished gonadotrophin response following GnRH administration should solely represent suppression by the contraceptive steroids. The response of the pituitary to a bolus dose of GnRH however, is also dependent on previous endogenous GnRH stimulation (also referred to as 'priming effect') (Wildt *et al.*, 1981a).

The prevention of ovulation in COC is thought to occur primarily by interfering with GnRH release (Schally *et al.*, 1970). Proof of this contention is brought about by the notion that the contraceptive effect of COC can be reversed by administering GnRH. Although GnRH administration should result in an increase of LH and FSH, levels are lower suggesting an inhibitory effect of COCs at both the hypothalamus and the pituitary level. A direct effect on the pituitary is also suggested since repeated GnRH administration does not always result in a LH and FSH response (De Leo *et al.*, 1991).

Pituitary suppression appears to be unrelated to age of the women and duration of COC use (Scott *et al.*, 1978a) but is dependent on the amount and type of progestin (Rommler *et al.*, 1982; Hemrika *et al.*, 1993) and the amount of EE (Scott *et al.*, 1978b). Furthermore, suppression of basal and GnRH stimulated gonadotrophin release is time dependent. I.e. lower doses need more time to establish suppression (Kuhl *et al.*, 1982; Kuhl *et al.*, 1984; Rommler *et al.*, 1985). The suppressive effect on basal and stimulated gonadotrophin release is also present in the first days of the pill-free period (Rubinstein *et al.*, 1978). Administration of several doses of recombinant FSH during high-dose COC use resulted in dose dependent follicle growth despite extremely low LH levels (Van Heusden *et al.*, 2002). Dominant follicles remained present following discontinuation of FSH and ongoing OC treatment suggesting that a direct effect, if any, of contraceptive steroids on dominant follicle growth is negligible. Once pre-ovulatory follicles are present during COC use, the administration of a either a GnRH analog (Elomaa and Lahteenmaki, 1999) or hCG (Killick, 1989) can induce ovulation.

2.6 Interpretation of pituitary-ovarian activity during steroid contraception

Many studies concerning pituitary-ovarian activity during steroid contraception are seriously devaluated in their relevance due to study design and/or interpretation of results. Infrequent (once per cycle) or conditional monitoring ('when a follicle of > 12 mm appears') in most studies allows for major underreporting of relevant events related to residual ovarian activity during steroid contraception. Despite early knowledge concerning the moment of ovulation in COC cycles, many studies focus on the second part of the cycle to assess ovulation. Obviously, the use of different assessments (urinary estrogens vs. serum E_2), hormone assays, sampling intervals and transvaginal or transabdominal ultrasound (Belaisch-Allart *et al.*, 1991) further reduce the possibility to compare results. Moreover, large intra- and inter-individual differences are present in contraceptive steroid serum levels, pharmacokinetics and pharmacodynamics (Jung-Hoffmann and Kuhl, 1990; Shaw *et al.*, 1992; Fitzgerald *et al.*, 1994). The origin of these differences and their consequences remain largely unexplained.

In several studies the occurrence of ovulation as a parameter of contraceptive efficacy is based upon serum P levels. Different cut-off levels are used: > 3 ng/ml (Kuhl *et al.*, 1985; Westcombe *et al.*, 1988), > 4 ng/ml (Chowdhury *et al.*, 1980), > 5 ng/ml (Rabe *et al.*, 1997) or > 16 nmol/l (Song *et al.*, 1993) and > 25 nmol/l (Fitzgerald *et al.*, 1994). However, the assessment of ovulation based on progesterone levels may be of limited significance in evaluating contraceptive properties of COC. A rise in P concentration in serum merely indicates luteal activity and hence is unable to discriminate between ovulation, premature luteinization or luteinized unruptured follicles. It is also speculative whether progesterone levels will always rise following ovulation during COC use. Progesterone production requires both FSH and LH, which may be suppressed to such an extend that normal steroid biosynthesis by the corpus luteum is compromised.

Classifications combining ultrasound assessments of follicle development (diameter, signs of ovulation) and hormonal parameters have been proposed (Landgren and Diczfalusy, 1980; Hoogland and Skouby, 1993; Van Heusden and Fauser, 1996) and used or modified by others (Fitzgerald *et al.*, 1994; Van der Does *et al.*, 1995; Spona *et al.*, 1996b). This method simplifies comparison between medications, studied cycles and individuals. A classification involves establishment of maximum ovarian activity during a studied cycle. However, relevant information is lost with regard to the timing of this event during the COC cycle. Furthermore,

some findings (e.g. when ultrasound assessments and endocrine assessment differ) remain unclassifiable (Barbosa *et al.*, 1990). Currently, there is no commonly accepted classification.

Benchmarking for different forms of steroid contraception requires the assessment of a combination of ultrasound and hormonal parameters, frequent sampling and a study design that allows for comparison and clinical application. We propose a design involving objective verifiable criteria for future use in studies with regard to pituitary-ovarian activity and COC use that allows for maximum clinical usefulness (Figure 3).

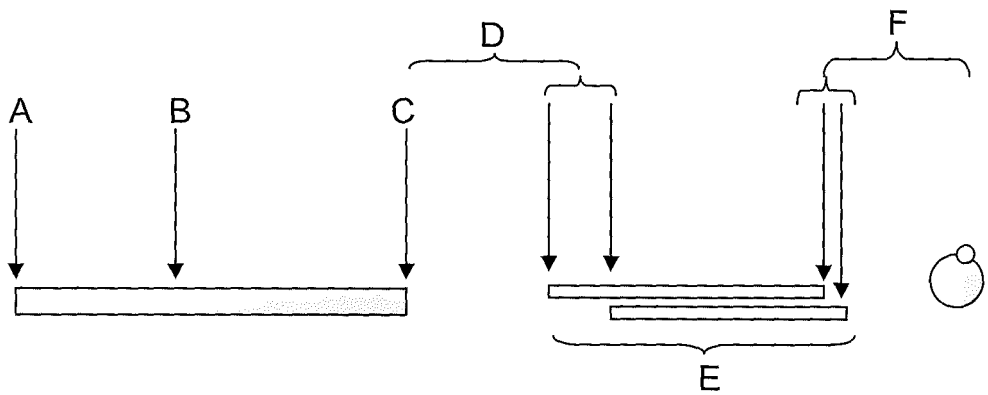


Figure 3 Proposal for uniform design in testing contraceptive effects on the pituitary-ovarian axis.

A: start of regular COC cycle: magnitude of pituitary-ovarian activity to control with studied COC

B: number of pill days to achieve maximum suppression (compare to C)

C: end of regular cycle; maximum suppression of pituitary activity expected

D: number of days for individual subjects to reach a follicle $\geq$ 11 mm

E: COC is started when follicle $\geq$ 11 mm; number of pill days until a follicle of $\geq$18 mm is observed

F: COC is discontinued when follicle of $\geq$ 18 mm is observed; number of days until ovulation occurs

2.7 Conclusions

The concomitant administration of oestrogens and progestins renders it difficult to evaluate the individual contribution of each entity with regard to suppression of the hypothalamic-pituitary-ovarian axis. However, effects of combined oral contraceptives have proven to be more than the sum of effects induced by either estrogens or progestins alone.

Many studies have been performed, but sub-optimal design or incomplete results often hamper comparison. No study design has yet evolved as a benchmark for contraceptive reliability. Although inhibition of follicle growth and ovulation is well understood, little is known with regard to the effects of contraceptive steroids on other systems controlling gonadotrophin secretion.

Chapter 3

Activity of the pituitary-ovarian axis in the pill-free interval during use of low-dose combined oral contraceptives

1 Introduction

The contraceptive effect during the use of combined oral contraceptives (COC) is predominantly established as a result of inhibition of the hypothalamic-pituitary-ovarian axis. Follicle growth is prevented and ovulation inhibited. The estrogen component is considered to inhibit FSH production and consequently diminish FSH-dependent follicle growth. Should, however, a dominant follicle emerge, inhibition of the LH surge and thus ovulation is prevented through the progestin component. The progestin component alone does not seem to have a prominent effect on basal concentrations of LH and FSH, but notably inhibits peak concentrations.(Kim Bjorklund *et al.*, 1992) Although not completely understood, inhibitory effects of both components in COCs are established through (synergistic) interactions at the hypothalamic-pituitary level. (Wan *et al.*, 1981; Wan *et al.*, 1981; Rommler *et al.*, 1982)

The seven-day pill-free period of most currently used regimens allows for withdrawal bleeding and serves the purpose of mimicking the normal menstrual cycle. In addition, it allows reduction of the overall amount of steroids administered over a four-week period. During the pill-free interval, pituitary-ovarian activity is allowed to resume in the absence of inhibitory steroids until the next medication strip is initiated. Numerous publications have described recovery of ovarian activity during the pill-free interval or following pill omissions.(Guillebaud, 1987; Fauser and Van Heusden, 1997) However, daily blood sampling together with ultrasound has rarely been performed. Few data are available to determine whether the magnitude of pituitary-ovarian suppression significantly differs among users of various low-dose COCs. The present study compared resumption of pituitary-ovarian activity in women using three different low-dose COCs to determine: (1) the maximum extent of suppression at the beginning of the pill-free interval, (2) the magnitude of recovery of pituitary-ovarian activity during the pill-free interval, and (3) the extent of pituitary-ovarian activity at the end of the pill-free interval, a starting point for the next cycle.

2 Subjects and Methods

Subjects and study protocol

Forty-four women using low-dose oral contraception were included in this single-center group comparative study in healthy female volunteers. The human ethics committee of the Dijkzigt Academic Hospital approved the study and all women gave written informed consent. The study was conducted according to the Declaration of Helsinki and the Good Clinical Practice (GCP) recommendations of the European Committee. Inclusion criteria were: age between 18-39 years, weight between 50 and 75 kg, and cyclelength between 24-35 days before starting the use of oral contraceptives. Excluded were women with hyperprolactinaemia or polycystic ovary syndrome, contraindications for the use of oral contraception or any relevant medical disorder. Each volunteer entered the study on the first day of the pill-free period following the correct use of at least two cycles of study medication.

Three low-dose COCs were used for comparison: Fifteen women used 20 μg EE + 75 μg gestodene (GSD) (Harmonet ®, Wyeth-Lederle, Hoofddorp, the Netherlands), 17 women used 20 μg EE + 150 μg desogestrel (DSG) (Mercilon ®, NV Organon, Oss, the Netherlands) and 12 women used 30 μg EE + 150 μg DSG (Marvelon ®, NV Organon, Oss, the Netherlands). Thirty-two women were randomly allocated to receive either 20 μg EE + 150 μg desogestrel or 20 μg EE + 75 μg gestodene . Each subject was assessed in pill-free period of the second cycle. The remaining 12 women using 30 μg EE + 150 μg DSG were enrolled while using the study medication for at least two months.

Assessments

All sonographic measurements were performed by a single investigator (AMvH) using a 6.5 MHz transvaginal probe (Hitachi, Tokyo, Japan) . Ovarian activity was assessed by counting the number of follicles after scanning each ovary from the inner to the outer margin in a longitudinal cross-section, as previously described.(Pache *et al.*, 1990), (van Santbrink *et al.*, 1995) The diameter was taken to be the mean of the size of the follicle in a longitudinal and an anteroposterior plane. Beyond a diameter of 10 mm, measurements in three planes were performed. Follicles >10 mm were considered dominant. (Pache *et al.*, 1990;van Dessel *et al.*, 1996) On every occasion, endometrial thickness was assessed as the maximum thickness (both sides) present in the longitudinal plane.

Serum samples were centrifuged within 2 hours after collection. Serum E_2 levels were measured by radioimmunoassay (Diagnostics Products Corporation, Los Angeles, USA). Serum

FSH and LH levels were determined by immuno-radiometric assay (Delfia kits, Kabi Pharmacia, Türkü, Finland). Intra-assay and interassay coefficients of variation (CVs) were <4.0 % and <6.4% for FSH, <15.5% and <14.1% for LH and <15% and <18% for E_2 , respectively. Samples from one individual were run in the same assay.

Data analysis

Results are presented as median and range unless stated otherwise. Study parameters were compared using the Mann-Whitney U-test, a non-parametric test for comparison of two independent groups. Differences were considered to reach statistical significance when $p<0.05$.

3 Results

All 44 volunteers completed the study. Age, weight and BMI were not statistically different among the study groups (data not shown). All women did use the studied COC for at least two months prior to the assessments in this study and there were no pill omissions reported. Figures 1-3 show the study parameters throughout the pill-free interval. Table 1 shows pituitary-ovarian activity at the beginning and end of the pill-free interval as well as parameters for the entire period.

Pituitary activity

In both 20 µg EE groups, a significant rise in serum FSH and E_2 was observed from day 3 of the pill-free period onwards ($p<0.01$, Wilcoxon Signed Rank test). In the 30 µg EE group, this occurred from day 4 onwards.

1 20 µg EE + 150 µg DSG versus 20 µg EE + 75 µg GSD

LH and FSH concentrations at the beginning (day 1) of the pill-free interval and at the end of the pill-free interval (day 7) were not significantly different. The area-under-the-curve (AUC) for both hormones was calculated as a measure for the total amount of hormone produced during this period. These were also not statistically significantly different.

2 20 µg EE + 150 µg DSG versus 30 µg EE + 150 µg DSG

Both LH and FSH concentrations were comparable on day 1. On day 7, FSH concentrations

were statistically higher in 30 μg EE + 150 μg DSG users (4.5 (2.4-7.4) IU/l versus 7.0 (0.6-12.4) IU/l; p=0.001). Despite the different shape of the FSH concentrations in both groups, the AUC for FSH and LH was not different.

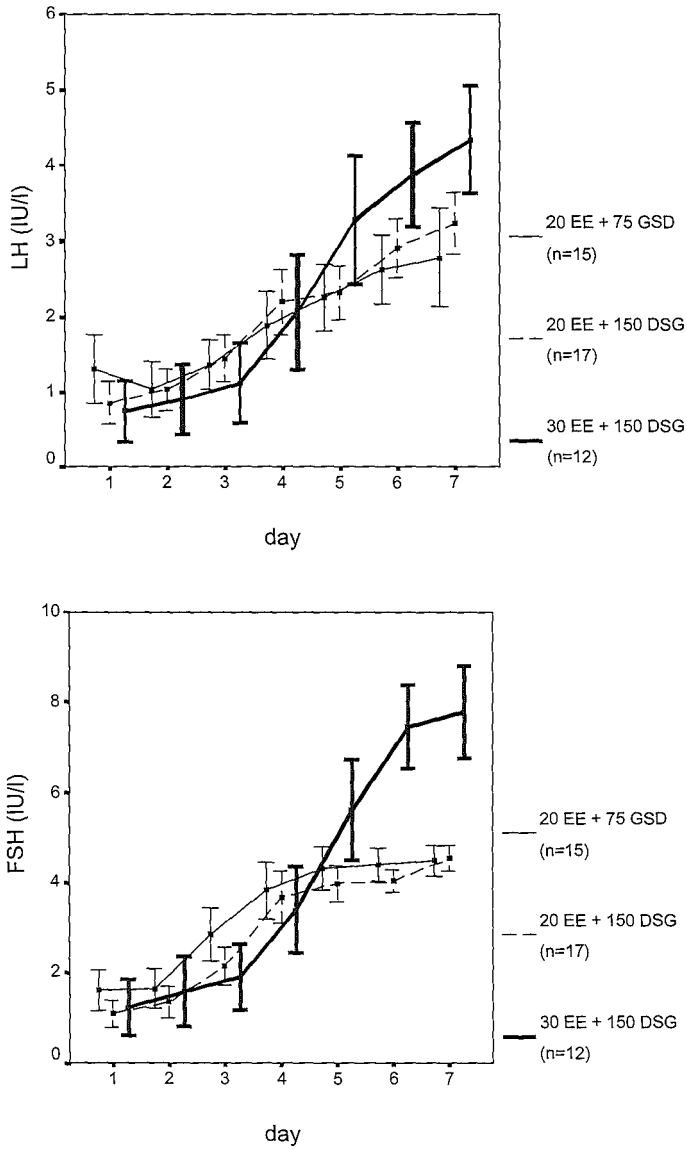


Figure 1

Daily serum concentrations (IU/l) of LH (upper panel) and FSH (lower panel) during the pill-free period (day 1-7) in 44 healthy volunteers using three different combined oral contraceptive regimens. Data are presented as mean ± SE.

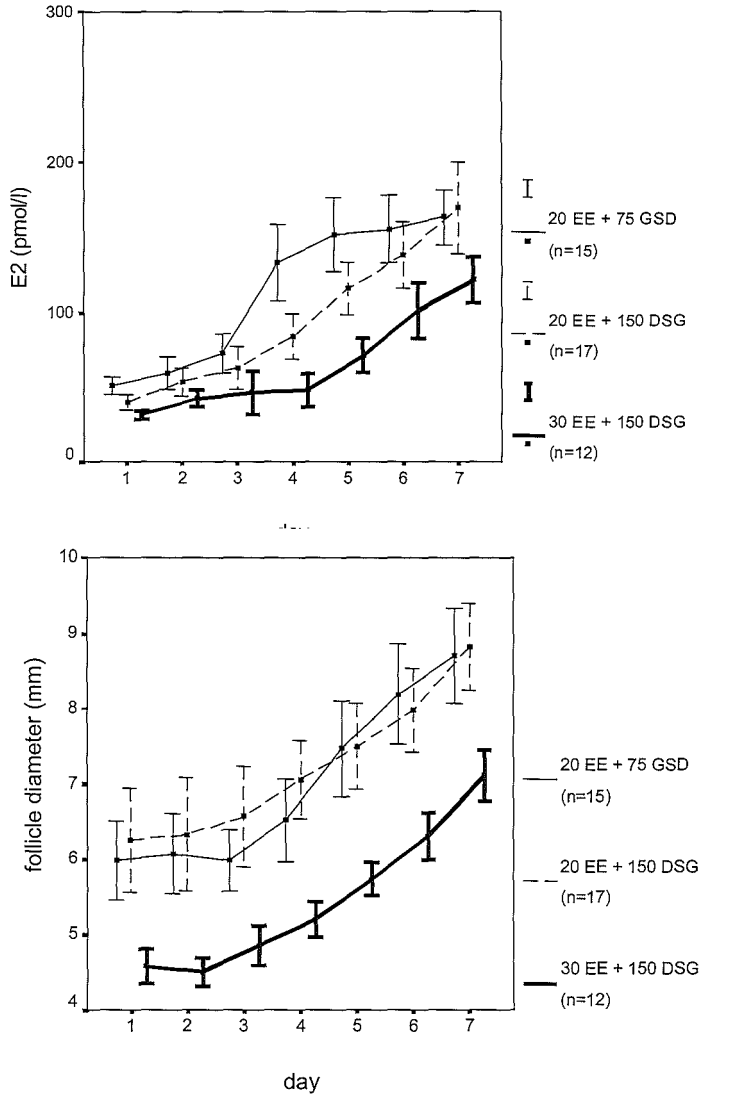


Figure 2

Daily maximum follicular diameters (mm) (left panel) and serum concentrations of E_2 (pmol/l) (right panel) during the pill-free period (day 1-7) in 44 healthy volunteers using three different combined oral contraceptive regimens. Data are presented as mean ± SE.

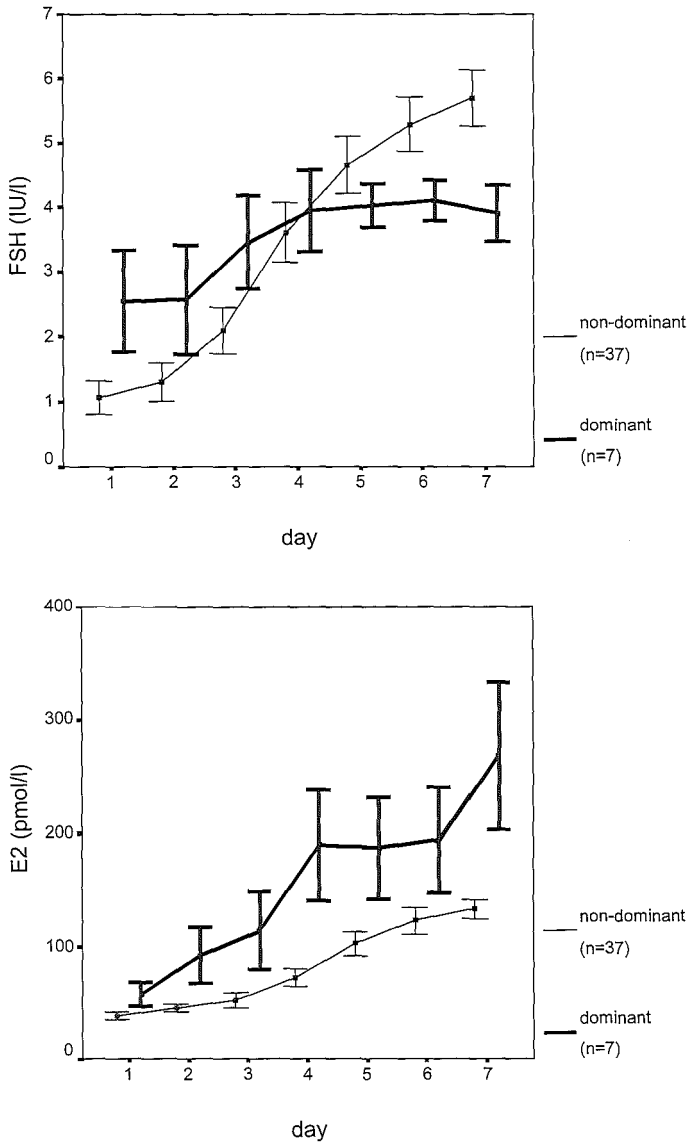


Figure 3

Daily serum concentrations of FSH (IU/l) (left panel) and serum concentrations of E_2 (pmol/l) (right panel) during the pill-free period (day 1-7) in women who have or do not have a dominant follicle present at the end of the pill-free period. Data are presented as mean $\pm$ SE.

Table 1

Endocrine and ultrasound characteristics during the pill-free period in three low-dose combined oral contraceptives users

Assessments		20 µg EE +75 µg GSD			20 µg EE + 150 µg DSG			30 µg EE + 150 µg DSG		
		Median / *range*		P*	Median / *range*		P**	Median / *range*		P***
LH	day 1	0.7	*0.05-6.2*		0.2	*0.05-3.7*		0.14	*0.05-4.6*	
(IU/l)	day 7	2.5	*0.05-11.0*		3.0	*0.3-6.5*		4.1	*0.1-9.6*	0.02
	AUC 1-7	12.5	*1.2-41.8*		10.3	*3.9-34.6*		12.9	*0.4-46.4*	
FSH	day 1	1.0	*0.05-5.4*		0.6	*0.05-3.9*		0.28	*0.05-6.1*	
(IU/l)	day 7	4.9	*1.4-6.1*		4.5	*2.4-7.4*	0.001	7.0	*0.6-12.4*	0.001
	AUC 1-7	22.6	*4.5-40.3*		20.9	*11.0-32.9*		26.5	*1.0-60.2*	
Maximum	day 1	5.6	*3.9-10.7* [1]		5.2	*4.0-15.2* [2]	0.01	4.5	*3.5-6.3*	0,05
follicle	day 7	7.9	*5.4-14.4* [1]		8.3	*5.5.-14.9* [2]	0.02	7.2	*5.2-9.0*	0.05
diameter	day1-7#	2.9	*-3.0-7.1*		2.6	*-0.3-4.7*		2.9	*-0.2-7.0*	
(mm)										
E_2	day 1	55	*12-97*		37	*14-90*		29	*20-52*	0.02
(pmol/l)	day 7	161	*82-315*		167	*68-622*		116	*43-214*	
	AUC 1-7	661	*343-1612*		567	*293-2153*	0,05	387	*295-907*	0.01

Area-under-the-curve (AUCs) were calculated for endocrine assessments; the increase of the maximum follicular diameter is given for sonographic assessments.

Statistical differences were calculated between group 1 and 2 (P*), group 2 and 3 (P**) and group 1 and 3 (P***).

#follicular growth from day 1 until day 7.

[1] in 1 patient, a persistent follicle was excluded (23.9 mm on day 1, 20.6 mm on day 7)

[2] in 1 patient, a persistent follicle was excluded (27.6 mm on day 1, 26.3 mm on day 7)

Ovarian activity

No ovulation was observed in either group. In both 20 µg EE study groups, one volunteer with a persistent follicle was seen throughout the pill-free interval, ranging from 23.9 mm to 20.6 mm in the 20 µg EE + 75 µg GSD group and 27.6 mm to 26.3 mm in the 20 µg EE + 150 µg DSG group. Since both structures were accompanied by low E_2 concentrations, they were omitted from comparative analysis and replaced by diameters from the second largest follicle present.

A follicle >10 mm was present at the end of the pill-free interval in 4 out of 15 women (27%) in the 20 μg EE + 75 μg GSD group group, in 3 out of 17 women (18%) in the 20 μg EE + 150 μg DSG, whereas in the 30 μg EE + 150 μg DSG group no follicles >10 mm were found. These differences were not statistically significant (Fisher's exact test).

1 20 μg EE + 150 μg DSG versus 20 μg EE + 75 μg GSD

Maximum follicle diameters did not differ statistically significantly at the start of the pill-free interval in the two 20 μg EE groups. Follicular growth of the largest follicle during the pill-free interval was similar in both groups: 2.9 mm (-3.0 - 7.1 mm) and 2.6 mm (-0.3 - 4.7 mm) for 20 μg EE + 150 μg DSG users and 20 μg EE + 75 μg GSD users, respectively. Finally, follicle diameters of the largest follicle present at the end of the pill-free interval were also not statistically different. E_2 concentrations did not differ statistically significantly on day 1 or day 7 between the two groups. The AUC for E_2 was also not different.

2 20 μg EE + 150 μg DSG versus 30 μg EE + 150 μg DSG

On day 1 of the pill-free interval, maximum follicle diameters were smaller in the 30μg EE + 150 μg DSG group, compared to the 20 μg EE + 150 μg DSG group (p=0.01). This difference was still present at the end of the pill-free interval (p=0.02). Follicle growth was not different in the two groups: 2.9 mm (-0.2 - 7.0 mm) and 2.6 mm (-0.3 - 4.7 mm) for 30 μg EE + 150 μg DSG and 20 μg EE + 150 μg DSG, respectively. E_2 concentrations did not differ on days 1 and 7 but the AUC for E_2 was just significantly higher in the 20 μg EE group (p=0.05).

The correlation between FSH concentrations and LH concentrations on day 1 of the pill-free interval was consistent throughout different medication groups (Pearson's r = 0.78, p=0.0001). However, other correlations were less powerful: E_2 and follicle diameter (Pearson's r = 0.49, p=0.001), FSH and E2 (Pearson's r = 0.34, p=0.02) and E_2 and LH (Pearson's r = 0.34, p=0.02). At the end of the pill-free interval, the strongest correlation was found between follicle diameter and E_2 concentration (Pearson's r = 0.61, p=0.001). Follicle growth correlated weakly with the AUC-FSH during the pill-free interval (Pearson's r = 0.32, p=0.03).

The presence of a dominant follicle at the end of the pill-free period was statistically significantly correlated with FSH on day 1 (p=0.05), E_2 on day 1 (p=0.05) and follicle diameter on day 1 (p=0.01), but not with the study medication or EE dosage.

4 Discussion

During the luteal-follicular transition of the normal menstrual cycle, FSH levels surpass the threshold for stimulating ovarian activity. This intercycle rise in FSH elicits recruitment of a cohort of synchronous follicles from which a single dominant follicle is selected later in the follicular phase of the cycle.(Fauser and Van Heusden, 1997)

Conventional combined oral contraceptives act primarily through inhibition of follicular growth in combination with peripheral progestin effects. In any situation in which medication is discontinued (either through 'missing the pill' or a scheduled cessation of medication in the pill-free interval), recovery of pituitary-ovarian activity has been documented. (Vallon *et al.*, 1992; Hedon *et al.*, 1992; Landgren and Csemiczky, 1991; Killick *et al.*, 1990; Smith *et al.*, 1986; Morris *et al.*, 1979) In the event of a scheduled seven day pill-free interval, pituitary-ovarian activity should remain suppressed to an extent that the development of dominant follicles is prevented. These follicles carry the risk of ovulation or continued growth to become persisting follicles/cysts. The inhibitory effect of the contraceptive combination should, therefore, be sufficient to arrest and repress the amount of activity present at the end of seven day pill-free interval. However, little is known about the influence of contraceptive combinations or the relative importance of the estrogen and progestin component on the dynamics of pituitary-ovarian recovery during the pill-free interval. The present study focused on pituitary ovarian activity during the pill-free interval with three low-dose COC's. By comparing Mercilon® (20 µg EE + 150 µg DSG) with either Marvelon® (30 µg EE + 150 µg DSG) or Harmonet® (20 µg EE + 75 GSD), the relative importance of the estrogen or progestin component was studied .

At the start of the pill-free interval, 20 µg EE-containing COCs are expected to have a lesser degree of pituitary suppression compared to the 30 µg EE COCs due to a dose-dependent effect on FSH secretion.(Goldzieher *et al.*, 1975) Although serum gonadotropin concentrations at the beginning of the pill-free interval were markedly reduced in the 30µg EE study group, no statistically significant differences were found in gonadotropin concentrations on the first day of the pill-free period between any of the study groups. This is probably largely due to inter-individual variations in gonadotropin concentrations and the relatively small sample size. The rate of pituitary recovery in the 30µg EE group appeared to be different compared to both 20 µg EE groups; the increase of LH and FSH started slower but was more pronounced towards the end of the pill-free interval. FSH concentrations in the 30 µg EE group were statistically higher compared to both 20 µg EE groups at the end of the pill-free period (see Table 1). It remains speculative whether the higher dosage of EE in the 30 µg EE group can be held

responsible for the continued FSH suppression during the first days of the pill-free period. Reduced ovarian feedback by hormones produced by early antral follicles such as E_2 and inhibin B may cause higher FSH levels at the end of the pill-free period in the 30 μg EE group.(Groome *et al.*, 1996)

Despite the lack of major differences in FSH and LH levels during the pill-free interval between the study groups, an important difference in ovarian activity was noted. In both study groups using 20 μg EE COCs, a persistent follicle >20 mm was observed at the beginning of the pill-free interval. Even when these were excluded from statistical comparison, maximum follicle diameters were significantly smaller in the 30 μg EE group. Growth rate of the maximum follicle present (as measured by the increase in maximum follicle diameter from day 1 to day 7) was not different between the study groups. No dominant follicles were seen in the 30μg EE + 150 μg DSG group, whereas 18-27% of women in both 20 μg EE group presented with dominant follicles. In three of these six women, dominant follicles were already present at the beginning of the pill-free period. The presence of a dominant follicle at the end of the pill-free period was statistically significantly correlated with FSH, follicle diameter and E_2 on the first day of the pill-free period. Also, FSH levels at the end of the pill-free interval in the 20 μg EE were lower. Figure 3 shows the different FSH and E_2 profiles for women who have or do not have a dominant follicle at the end of the pill-free interval. It appears that the lower FSH levels at the end of the pill-free period might result from endogenous E_2 feedback mechanisms if dominant follicles are present.

Estradiol levels were not statistically different either at the beginning or the end of the pill-free interval among the study groups, although the AUC for E_2 was statistically higher in the 20 μg EE groups. Combined with follicular measurements, it appears that ovarian suppression is generally less during the pill-free interval in the 20 μg EE groups.

Since FSH concentrations and follicular growth rates during the pill-free interval do not differ between the 20 μg EE and 30 μg EE study groups, it is the follicular diameter at the beginning of the pill-free interval that probably provides the risk for selection and dominance. Once dominant follicles emerge, FSH levels will decline due to endogenous feedback mechanisms. This could explain the lower FSH levels in the 20 μg EE groups in this study. The more follicles mature during the pill-free period, the less FSH they need to continue development during the next cycle (i.e., enhanced sensitivity for FSH of the maturing follicle). In other words, the more follicles are allowed to develop during the pill-free period the more EE (and subsequent FSH suppression) is required thereafter. A suitable measure in that case would be a reduction of the length of the pill-free interval. Of course, well-known inter-individual

differences exist in metabolism of contraceptive compounds (Bazin *et al.*, 1987) and may indeed explain a reduction in control of pituitary-ovarian activity in many cases.

In this study, modest differences in endocrine parameters among the three studied populations were found. Inter-individual differences within a group often exceeded those between the groups. Although samplesize may prevent the achievement of statistical significance in some instances, the differences in follicle diameter may be clinically relevant. The present data suggest that a decrease in the EE content as seen in the 20 µg EE-containing COCs result primarily in larger follicles during the pill-free interval. Since follicles maintain the potential to ovulate, (Killick, 1989) contraceptive efficacy in COC should include the prevention of dominant follicles.

Chapter 4

FSH and ovarian response: Spontaneous recovery of pituitary-ovarian activity during the pill-free period versus exogenous recombinant FSH during high-dose combined oral contraceptives.

1 Introduction

The manipulation of follicular growth remains the focus of ongoing clinical research aiming at establishing new regimens for the inhibition or stimulation of ovarian activity (Fauser and Van Heusden, 1997). Inhibition of ovulation is the cornerstone of conventional combined oral contraception. The lowest possible amount of exogenous steroids consistently inhibiting ovulation, while maintaining desirable bleeding patterns is sought. When stimulating follicle growth for assisted reproduction, exogenous FSH, human chorionic gonadotrophin (hCG) and gonadotrophin releasing hormone (GnRH) analogues are used in different regimens and combinations for (controlled) ovarian hyperstimulation (Macklon and Fauser, 2000).

Combined oral contraceptive (COC) steroids affect gonadotrophin secretion through direct pituitary suppression (Mishell *et al.*, 1977; Dericks-Tan *et al.*, 1983; De Leo *et al.*, 1991). Complete hypothalamo-pituitary-ovarian inhibition with high-dose COC's provides a good model to study the ovarian response to exogenously administrated FSH and seems comparable to initial studies with recombinant FSH in hypogonadotrophic hypogonadal women (Schoot *et al.*, 1992;Schoot *et al.*, 1994). Thus exogenously administered FSH is compared with the effect of endogenously produced FSH and LH during the pill-free interval. During the 7-day pill-free period, the inhibitory effect of the contraceptive steroids diminishes and folliculogenesis recurs. If resumption of FSH secretion is prompt and serum levels rises above the FSH-threshold (Brown, 1978) the FSH window (Baird, 1987; Fauser *et al.*, 1993) is opened to allow for the cyclic recruitment of follicles (McGee and Hsueh, 2000). The start of a subsequent COC cycle will lower FSH levels and close the FSH window. Previous studies on hypothalamo-pituitary-ovarian activity during the pill-free period indicated that GnRH pulsatility resumes completely (Van der Spuy *et al.*, 1990) and dominant follicles beyond 9 mm diameter (van Santbrink *et al.*, 1995) can emerge at the end of this interval (Doyen *et al.*, 1987; Hamilton and Hoogland, 1989; Grimes *et al.*, 1994). In this respect, the pill-free period mirrors the intercycle rise in FSH (Van Heusden and Fauser, 1996).

FSH preparations are often combined with GnRH agonists to suppress endogenous pituitary activity in ovarian hyperstimulation protocols for in-vitro fertilization. Although COC's have been given prior to administration of GnRH analogs to allow planning of ovum pick-up procedures (Cohen *et al.*, 1987), they can also be used to suppress endogenous pituitary activity while studying the effects of FSH preparations (Out *et al.*, 1996). The current study was designed to compare spontaneous pituitary-ovarian activity during the pill-free period with ovarian stimulation using different doses of exogenous FSH during suppression of endogenous

gonadotrophin release by a high dose COC.

The extent of ovarian response to different dosages of exogenous FSH during continued high-dose COC suppression is compared with spontaneous recovery of ovarian activity during the pill-free period after low-dose COC.

2 Subjects and methods

Subjects

Thirty-six women using low-dose (i.e. < 35 mcg ethinyl-estradiol) oral contraception were recruited to participate in this single-center, group-comparative, randomized study in healthy volunteers. The protocol was approved by the local human ethics review committee and all women gave written informed consent. Women received financial compensation for their participation. The study was completed according to the guidelines of Good Clinical practice (GCP) and conducted in full compliance with the World Medical Association Declaration of Helsinki. Inclusion criteria were age between 18-39 years, pre-study oral contraceptive use for at least 3 months, cycle length between 24-35 days before the use of oral contraceptives and body weight between 50 and 75 kg. Women with hyperprolactinaemia, polycystic ovary syndrome, contra-indications for the use of gonadotrophins or oral contraception or any other relevant medical disorders were excluded.

Medication and study design

All 36 volunteers entered the study on the first day of the pill-free period following the correct use of the pre-study COC (Figure 1). Following the 7 day pill-free interval, the combined oral contraceptive Lyndiol® (2.5 mg lynestrenol + 0.05 mg ethinyl-oestradiol; NV Organon, Oss, the Netherlands) was started for a period of 5 weeks. In the 4th week of Lyndiol® use, volunteers received either 75 IU recombinant FSH (recFSH, Puregon®; NV Organon; n=9), 150 IU recFSH (n=9), 225 IU recFSH (n=9) or 150 IU purified urinary FSH (uFSH, Metrodin®; Serono, Geneva, Switzerland) (n=9) for a period of 7 days. Subjects were randomly assigned to 1 of the treatment groups by allocating a subject code by means of a sealed envelope. All FSH medication was given daily as a single intramuscular injection following blood sampling. FSH immunoreactivity of the batch of uFSH ampoules was 77.8 IU/ampoule and of recFSH 64.4 IU/ampoule. Subjects included in this study received FSH from the same batch.

During the 6 week study period, assessment of pituitary-ovarian activity (transvaginal

ultrasonography and blood sampling) was performed on days 1 to 10, 15, 22, 28 to 42, 45, and 49 (Figure 1).

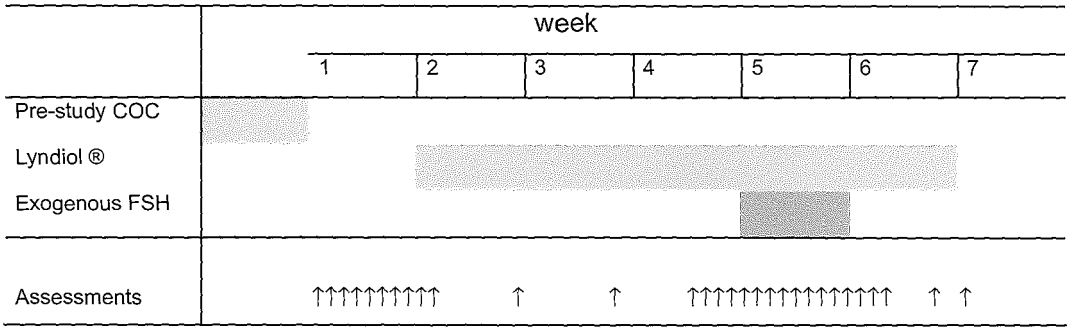


Figure 1 Study schedule

Assessments

All sonographic assessments were performed by a single investigator (AMvH) using a 6.5 Mhz transvaginal probe (EUB-415, Hitachi Medical Corporation, Tokyo, Japan). Follicular growth was assessed by counting the number of follicles after scanning each ovary from the inner to the outer margin in a longitudinal cross section, as previously described (Pache *et al.*, 1990; van Santbrink *et al.*, 1995). All follicles beyond 2 mm diameter were counted and registered. The diameter was taken to be the mean of the size of the follicle in a longitudinal and an antero-posterior plane. Beyond a diameter of 9 mm, measurements in 3 planes were performed. Follicles > 10 mm were considered dominant (Pache *et al.*, 1990; van Santbrink *et al.*, 1995; van Dessel *et al.*, 1996). On every occasion, endometrial thickness was assessed as the maximum thickness (both sides) present in the longitudinal plane.

Bloodsamples were processed (centrifuged at 2000 G (20.000 N/kg) for 15 minutes) within 120 minutes after venepuncture and frozen (-20 C) for storage until assayed. Serum E_2 and P levels were estimated by radioimmunoassay (Diagnostics Products Corporation; Los Angeles, USA) (De Jong *et al.*, 1974). Serum FSH and LH levels were determined by immunoradiometric assays (Medgenix, Fleurus, Belgium) (Fauser *et al.*, 1991). Intra- and interassay coefficients of varation (CV's) were < 3 % and < 8% for FSH, < 5% and < 15% for LH, <16% and 17<% for P and <15% and 18<% for E_2 respectively.

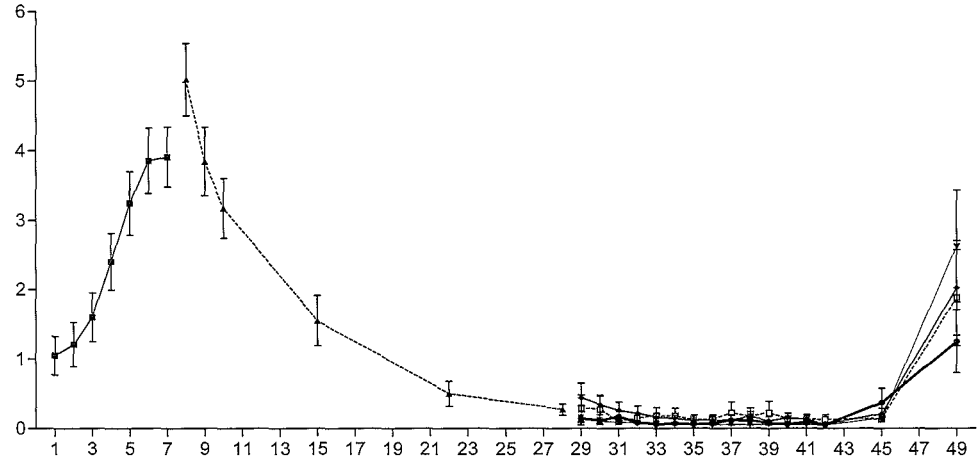
Data analysis

Results are presented as mean and standard deviation unless stated otherwise. Study parameters were compared using the Mann-Whitney U test. Spearman correlation coefficients (rs) were used to describe correlations. Means were compared using the independent t-test, or, where appropriate, the Yates test. Differences were considered to reach statistical significance when $P < 0.05$.

3 Results

All 36 subjects completed the study. Relevant demographic data (length, weight, age and duration of pre-study COC use) and endocrine parameters (dehydroepiandrosterone sulphate, testosterone and prolactin) obtained at screening were comparable for all study groups (data not shown). In Figure 2, pituitary-ovarian activity during the study period is displayed. Figure 3 displays the area under the curve (AUC) during the first 8 days for FSH, LH and E_2 during the pill-free period and during exogenous FSH administration. The increase in maximum follicular diameter and changes in follicle number is also shown.

LH (IU/l)



FSH (IU/l)

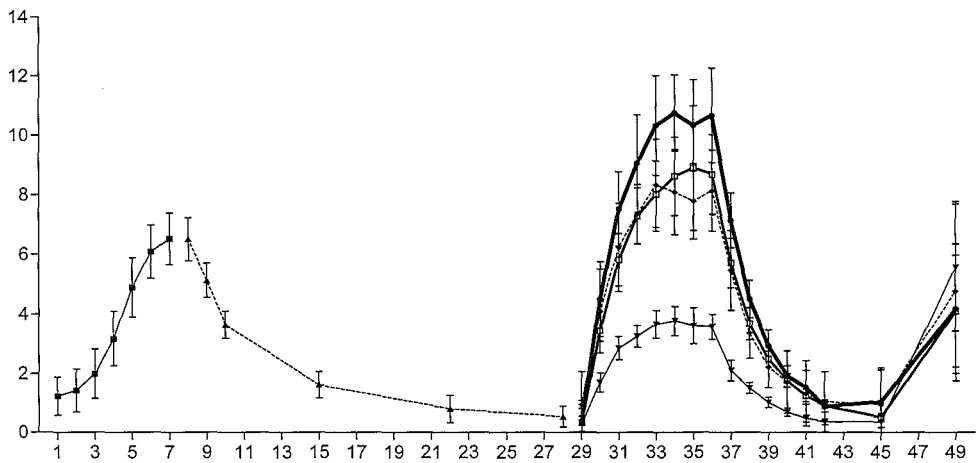


Figure 2 Pituitary-ovarian activity (mean ± 95% CI) during the study period.

Day 1 - 7 : pill-free period following the pre-study COC. Day 8 - 28: the first 3 weeks of high-dose COC use (representing pill-day 1-3, 7, 14 and 21 respectively). Day 29 – 35: week of exogenous FSH administration. Day 36-42: subsequent week during high-dose COC use. Day 43-49: second pill-free period following cessation of all study drugs.

— 75 IU recFSH — 150 IU recFSH — 225 IU recFSH ····· 150 IU uFSH

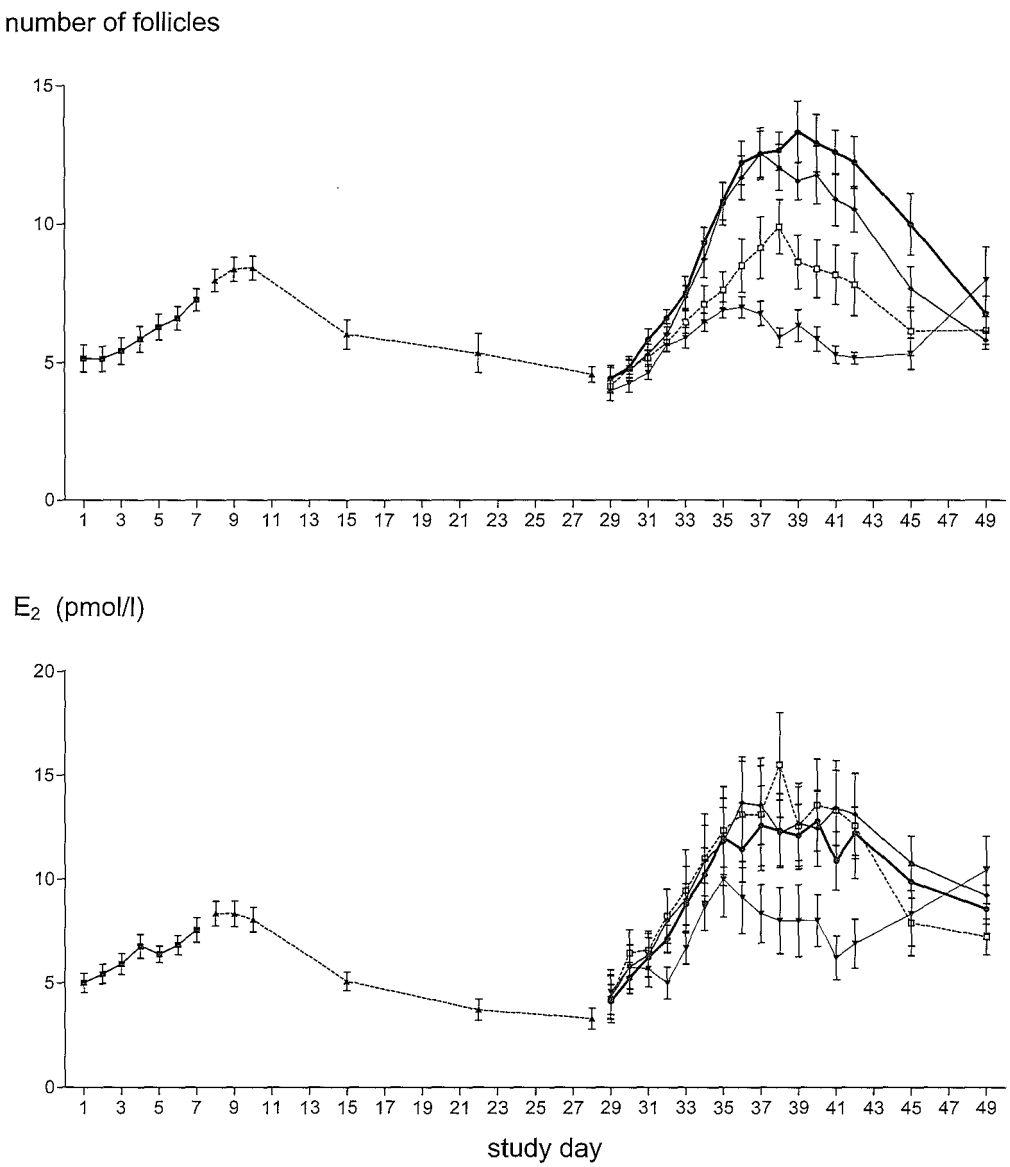
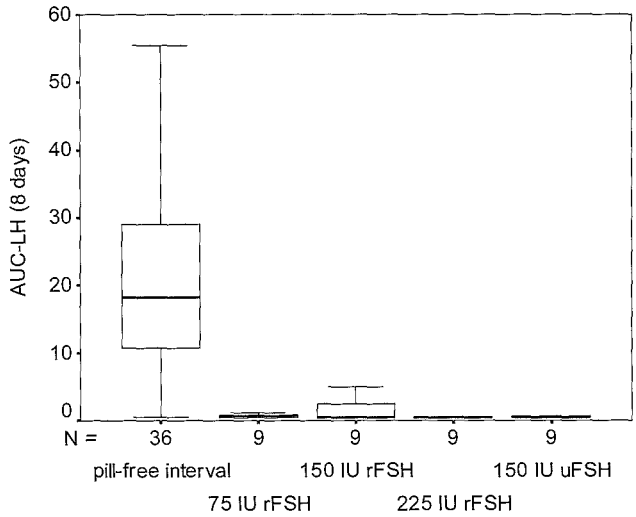


Figure 2 *(continued)* Pituitary-ovarian activity (mean ± 95% CI) during the study period.

Day 1 - 7 : pill-free period following the pre-study COC. Day 8 - 28: the first 3 weeks of high-dose COC use (representing pill-day 1-3, 7, 14 and 21 respectively). Day 29 – 35: week of exogenous FSH administration. Day 36-42: subsequent week during high-dose COC use. Day 43-49: second pill-free period following cessation of all study drugs.

— 75 IU recFSH — 150 IU recFSH — 225 IU recFSH ····· 150 IU uFSH



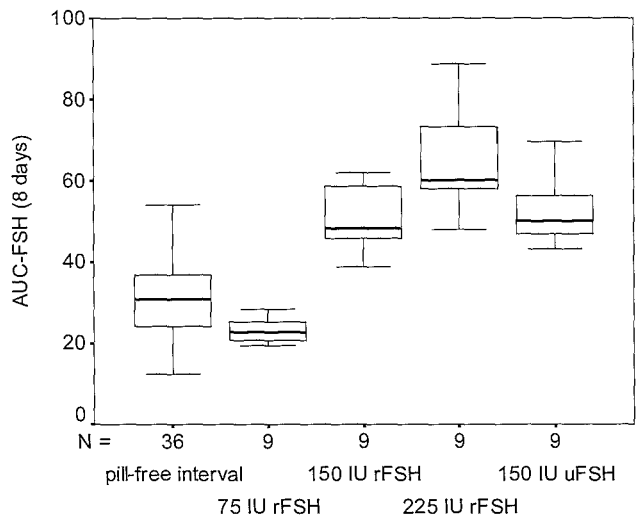


Figure 3

Area-under-the-curve plots for LH and FSH for day 1-8 during the pill-free period and during FSH administration.

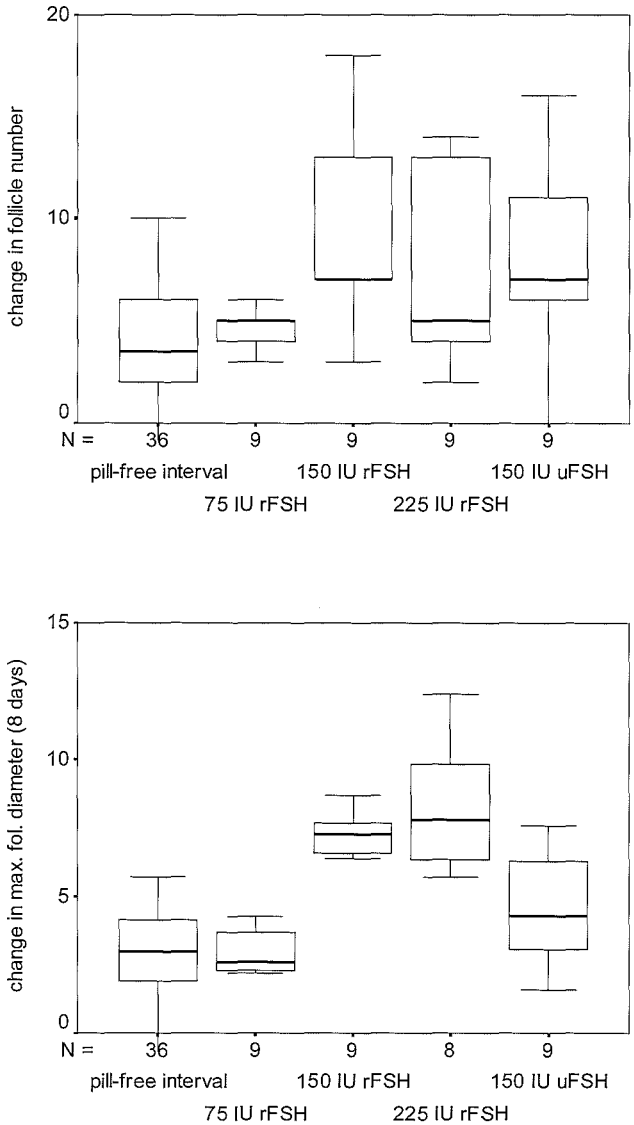


Figure 3 *(continued)*

Change in follicle number and change in maximum follicle diameter during during the pill-free period and during FSH administration.

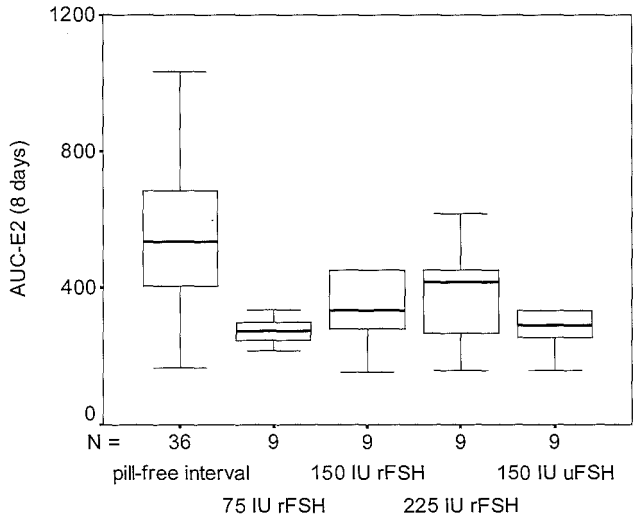


Figure 3 *(continued)*

Area-under-the-curve plot for E_2 for day 1-8 during the pill-free period and during FSH.

Pituitary-ovarian activity during the pill-free period (day 1-7)

On the first day of the pill-free period, both LH and FSH were suppressed considerably. In 24/36 volunteers (67%) both LH and FSH were < 1 IU/l. Both LH and FSH on the first day of the pill-free period correlated ($P < 0.01$) with maximum LH levels, maximum E_2 levels and maximum follicle diameters but not with maximum FSH levels. Initial and maximum follicle number as well as the initial and maximal E_2 levels were statistically significantly correlated ($P < 0.01$). At the start of the pill-free period, a single follicle of 20 mm was seen (E_2 = 47 pmol/l). This follicle did not rupture during the pill-free interval but disappeared gradually during the use of the study COC.

Endocrine parameters (FSH = 6,5 $\pm$ 2.6 IU/l; LH = 3,9 $\pm$ 2.6 IU/l; E_2 = 123 $\pm$ 60 pmol/l) at the end of the pill-free period were comparable with the mid-follicular phase of a normal menstrual cycle (Fauser and Van Heusden, 1997).

Pituitary-ovarian activity during the first 3 weeks of high-dose COC use (day 8-28)

Following 7 days of Lyndiol® use (day 15), no statistical differences were found in levels for LH, FSH, follicle diameter and E_2 compared to the beginning of the pill-free period.

Only a single dominant follicle was seen on the first day of Lyndiol® intake. However, on the third day of study COC use, seven volunteers presented with a total of 10 follicles > 10 mm. Only one of these follicles continued to grow and reached a diameter of 20 mm on the 7th day of study COC use. This follicle was still present on the 14th day of study COC use.

Following 3 consecutive weeks of study COC use (day 28), levels of LH and FSH were significantly more suppressed compared to levels found after 3 weeks of low-dose COC ($P = 0.007$ and $P = 0.005$ respectively). Maximum follicular diameter and E_2 levels were comparable but the number of follicles was significantly lower following Lyndiol® use ($P = 0.006$).

Exogenous FSH administration during continued high-dose COC use (day 29 –42)

LH levels remained profoundly suppressed (mean LH <0.5 IU/l in all subjects) and did not differ among the 4 studied groups. The AUC's for LH (Figure 3) were comparable between the FSH groups, while the AUC during the pill-free period was significantly higher ($P = 0.001$).

FSH levels obtained during FSH administration were dose dependent. The ratio for their AUC's was 75 recFSH : 150 recFSH : 150 uFSH : 225recFSH = 1,0 : 2,25 : 2,24 : 2,76. The AUC for FSH during the pill-free period following low-dose COC appears to be comparable with daily administration of 75-150 IU recFSH.

Growth of the leading follicle in the first 8 days (Fig 3) differed significantly between the studied groups except between 150 IU recFSH and 225 IU recFSH. The increase in maximum diameter and growth rate during the pill-free period was comparable with the 75 IU recFSH and 150 IU uFSH group (despite distinct differences in LH and FSH concentrations), but significantly lower compared to the 150 IU recFSH or 225 IU recFSH group. In Figure 4, all follicles were ordered according to their diameter and counted to visualize the dynamics of follicle growth. There were no significant differences in the number of follicles.

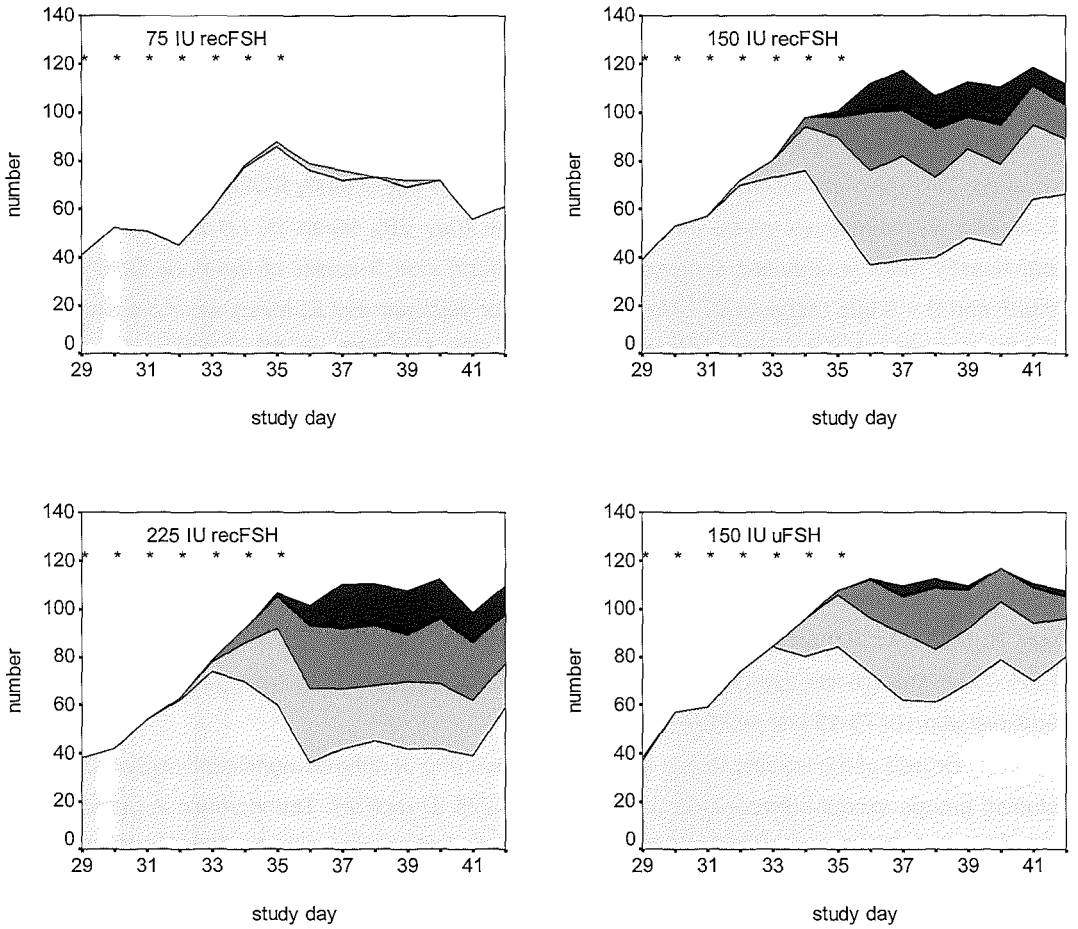


Figure 4

Follicle number categorized by diameter during the week of exogenous FSH administration * (day 29-35) and subsequent week (day 36-42) during use high-dose COC.

Follicle diameter : < 8mm 8-10 mm 10-12 mm >12 mm

Finally, both the maximum E_2 level and the AUC for E_2 during the first 8 days (fig 4) was not significantly different amongst the studied groups but in all groups it was lower compared to the pill-free interval.

A statistically significant correlation was observed between the AUC for LH and the AUC for E_2 during the pill-free period ($p = 0,001$) and in the 150 IU recFSH group ($p = 0.046$) while daily serum concentrations of LH and E_2 correlated in all groups except in the 225 IU recFSH group.

4 Discussion

We describe pituitary-ovarian activity during the pill-free period in 36 women using low-dose COC and the ovarian follicle development following the administration of different dosages of exogenous FSH during continued administration of a high-dose COC to suppress endogenous gonadotrophins.

During the pill-free period following the correct use of the pre-study COC, recovery of the pituitary-ovarian axis is comparable to what has been described earlier (Van der Spuy *et al.*, 1990; Tayob *et al.*, 1990; Van Heusden and Fauser, 1999). Gonadotrophin levels on the first day of the pill-free period appeared to be a marker for the magnitude of resumption of ovarian activity at the end of the pill-free period.

A de novo dominant follicle was observed in only a single subject at the end of the pill-free period. However, 7 volunteers (19%) presented with > 1 dominant follicle during the first days of study COC use. This is in line with other studies where follicle growth beyond 9 mm at the end of the pill-free period was found in approximately 20-30% of COC users (Fauser and Van Heusden, 1997). Although some follicles continued to grow past the first days of study COC use, follicle diameters after 7 days of high-dose COC were comparable to day 1 of the pill-free period. This finding is consistent with previous observations that 7 days of COC suffice to suppress follicle growth to levels comparable with those found at the beginning of the pill-free period (Guillebaud, 1987). Persisting follicles, a common feature in COC, seem to emerge from dominant follicles which develop at the end of the pill-free period. Those follicles which continue to grow during COC use and retain their potential for ovulation (Killick, 1989). However, due to the almost immediate suppression of LH secretion, ovulation is unlikely.

The administration of various doses of exogenous FSH for 7 days during the continued

use of Lyndiol® resulted in a dose-dependent increase in serum FSH levels. Statistically significant differences were observed between the studied groups regarding maximum FSH levels and AUC for the period of FSH administration. Compared to rising FSH concentrations during the pill-free period, maximum FSH levels were higher than the 75 IU recFSH group, comparable with 150 IU recFSH or 150 IU uFSH and lower than the 225 IU recFSH group. The AUC for FSH during the pill-free period was higher than 75 IU recFSH but lower for all other groups. LH levels remained suppressed at very low concentrations during Lyndiol© use and exogenous FSH administration. No statistical significant differences were observed in LH levels on any day, maximum LH levels or the AUC for LH during FSH administration between study groups. Follicle growth was seen in all study groups. This seems to corroborate the hypothesis that contraceptive steroids do not have a direct inhibitory effect on follicle growth. Instead suppression of follicle growth is achieved through suppression of FSH. Furthermore, follicle growth is observed despite extremely low LH levels in line with previous observations in hypogonadotrophic women receiving recFSH (Schoot *et al.*, 1992) . According to the two-cell two-gonadotropin theory both FSH and LH are required for adequate biosynthesis of E_2. Despite an (FSH-dose related) increase in number and diameter of follicles during exogenously administered FSH, maximum E_2 levels and the AUC for E_2 did not differ comparing different doses of exogenous FSH. Compared with the pill-free interval, both maximum E_2 levels and the AUC for E_2 were significantly lower in all groups. It thus appears that the low endogenous LH levels due to the use of high-dose COC limit the synthesis of E_2 during FSH administration. These observations substantiate earlier findings in the human suggesting that E_2 concentrations are not required for dominant follicle growth (Fauser and Van Heusden, 1997), (Palter *et al.*, 2001). It should, however, be noted that expansion of a follicle-like structure as assessed by ultrasound not necessarily indicates true optimal follicular development.

It has been emphasized recently that LH is crucial for optimal follicle growth (Filicori, 1999). Appropriate LH bio-activity is also required for oestrogen mediated endometrial development and cervical mucus characteristics. However, in the current study follicle growth was seen in proportion to the administered dose of FSH despite the very low LH levels. The total number of counted follicles with a diameter of $\geq$ 3 mm was remarkably similar during FSH administration in all groups. Apparently, the FSH threshold in all groups is readily surpassed and appears to be quite similar for FSH dosages above 75 IU. However, the possibility cannot be excluded that the number of follicles responding to FSH is affected by the lack of LH brought about by high-dose COC.

In summary, FSH suppression remains the cornerstone of COC efficacy. The pill-free interval in low-dose COC allows dominant follicles to emerge due to recovery of pituitary activity.

These follicles continue to grow during the first days of COC intake. Once dominant follicles emerge they may even reach pre-ovulatory diameters albeit with low E_2 levels. The 'functional life-span' (Faundes *et al.*, 1996) (sonographically detectable increase in follicle diameter and concomitant E_2 production) of dominant follicles is therefore short. Exogenous FSH administration during high-dose COC induced (dominant) follicle growth which remained remarkably stable in the week thereafter. This is in contrast to the almost complete suppression of (non-dominant) follicular activity after 1 week of high dose COC following a regular pill-free period. Hence, dominant follicles are more difficult to control with COC due to their relative independence of FSH and this follicle development occurred despite very low levels of LH and E_2.

Chapter 5

Single monthly administration of the Anti-progestagen Org 31710 in users of the 75 µg desogestrel progestagen-only-pill: Effects on pituitary - ovarian activity

1 Introduction

Insufficient cycle control represents the major problem associated with any progestagen-only-contraceptive regimen (Belsey, 1988; Broome and Fotherby, 1990; Shoupe *et al.*, 1991). Both in progestagen-only-pills (POP) and in progestagen-only delivery systems (medicated IUD's, injectables, vaginal rings and implants) frequent and unpredictable bleeding is often present. This may be caused by a direct effect of the progestagen on the endometrium leading to atrophy or through the incomplete suppression of pituitary-ovarian activity.

Hodgen and colleagues demonstrated in monkeys that the anti-progesterone mifepristone could induce amenorrhoea by inhibiting oestrogen-induced endometrial proliferation (Van Uem *et al.*, 1989). Low dose mifepristone induced endometrial secretory transformation, but higher doses inhibited proliferation and secretory activity. In levonorgestrel - treated monkeys, mifepristone co-administration inhibited the secretory transformation of the endometrium by progestagens (Wolf *et al.*, 1989b). In the presence of oestradiol (E_2) both mifepristone and onapristone were able to increase the number of endometrial E_2 and progesterone (P) receptors (Neulen *et al.*, 1996). Thus supplementary administration of anti-progesterone to a progestagen-only-contraceptive regimen might improve cycle control possibly as a result of blocking progestagen receptors on the endometrium (Hodgen *et al.*, 1994; Heikinheimo *et al.*, 1996). The combination of a POP and an anti-progestagen may yield a new type of oestrogen-free oral contraceptive, with improved bleeding characteristics compared to the classical POP.

Org 31710 is a strong anti-progestagen with low anti-glucocorticoid activity and weak androgenic / anti-androgenic and anabolic properties (Mizutani *et al.*, 1992; Kloosterboer *et al.*, 1994). The present study was designed to assess the effects of Org 31710 or a placebo on pituitary-ovarian activity and the effects on endometrial thickness during a continuous oral 75 µg desogestrel (DSG) progestagen-only regime.

2 Subjects and methods

Study design and subjects

A phase II, double blind, placebo-controlled two-centre study was conducted to investigate the effects of 150 mg Org 31710 given once per 28 days on serum levels of Follicle Stimulating

Hormone (FSH), Luteinizing Hormone (LH), E_2 and P, follicular diameters and endometrial thickness assessed by transvaginal sonography in healthy, female subjects using a 75 µg DSG POP. This two-centre study which was performed as part of a larger multi-centre study (n=104; 6 centres) assessing the safety and probable efficacy of the study medication in improving cycle control.

The Ethics Committee of both centres approved the study. The study was completed according to the guidelines of Good Clinical Practice and conducted in full compliance with the World Medical Association Declaration of Helsinki. Each subject gave written informed consent before participation.

Inclusion criteria were age between 18 and 45 years, good physical and mental health, cycle length between 24 and 35 days (with an intra-individual variation of ± 3 days), Body Mass Index between 18 and 29 kg/m2 and the use of barrier methods of contraception during the entire study period. Excluded were women either lactating or within a period of two months after a delivery or abortion, abnormal haematological or biochemical values at screening, PAP smear class III or higher, undiagnosed vaginal bleeding, use of an injectable hormonal contraceptive within 6 months prior to the start of study or any other hormonal contraceptive within 4 weeks prior to the study. Subjects were randomly assigned to one of the treatment groups by allocating a subject code.

Medication

DSG was given as oral tablets of 75 µg for 196 days (7 cycles of 28 days). Org 31710 was supplied as oral tablets of 50 mg. Three tablets (or identically appearing placebos), were given on days 1, 28, 56, 84, 112, 140 and 196 in a double blind fashion.

Assessments

Pituitary-ovarian activity was assessed every other day during the third cycle in one centre (Centre NL) and at least twice weekly during the fourth or fifth cycle in the other centre (Centre UK).

A single investigator in each centre using a 7.5 MHz transvaginal probe performed all sonographic measurements. Follicular activity was assessed by means of vaginal ultrasound counting the number of follicles after scanning each ovary from the inner to the outer margin in a longitudinal cross-section, as previously described (Pache *et al.*, 1990; van Santbrink *et al.*, 1995). The diameter was taken to be the mean of the size of the follicle in a longitudinal and an antero-posterior plane. Beyond a diameter of 10 mm, measurements in 3 planes were

performed. Follicles > 10 mm were considered dominant (Pache *et al.*, 1990; van Santbrink *et al.*, 1995). On every occasion, endometrial thickness was assessed as the maximum thickness (both sides) present in the longitudinal plane. Serum samples were centrifuged within 2 hours of withdrawal. Serum E_2 levels were measured by radioimmunoassay (Diagnostics Products Corporation; Los Angeles, USA). P levels was determined by RIA as described before (De Jong *et al.*, 1974). Serum FSH and LH levels were determined by immuno-radiometric assay (Delfia kits, Kabi Pharmacia, Türkü, Finland). Intra-assay and inter-assay coefficients of variation (CV's) were < 4.0 % and < 6.4% for FSH, < 15.5% and < 14.1% for LH and <15% and <18% for E_2 and <16 and < 17% for P respectively. Samples from one individual were run in the same assay.

Data analysis

Results are presented as mean and 95% confidence interval unless stated otherwise. Study parameters were compared using the Mann-Whitney U test. Differences were considered to reach statistical significance when $p < 0.05$.

3 Results

Demographics

Two women were excluded from analysis due to protocol violations (both placebo). All remaining 48 volunteers (25 Org 31710, 23 placebo) completed the study. Relevant demographic parameters were not statistically different between the two medication groups (age 29.2 $\pm$ 6.0 yr vs 29.1 $\pm$ 5.4 yr, weight 65.5 $\pm$ 9.5 kg vs 65.6 $\pm$ 10.2 kg, height 167.3 $\pm$7.8 cm vs 168.0 $\pm$ 5.3 cm; Org 31710 (n=25) vs placebo (n=23), mean $\pm$ SD).

Pituitary-ovarian activity

Forty-eight women were included in the evaluation of pituitary activity. Summary statistics for FSH, LH, E_2, P and follicle diameter are presented in Table I.

Table I

Endocrine and ultrasound parameters of pituitary-ovarian activity during Org 31710 vs placebo in users of 75 µg desogestrel progesterone–only pills. Data (mean + SD) reflect both the entire monitored cycle from day 1-28 (marked 1-28) and the maximum value (Max.) during that cycle.

		Org 31710 (n=25)	**Placebo** (n=23)	P value
FSH (IU/l)				
	1-28	4.5 ± 1.8	4.7 ± 1.7	
	Max	7.3 ± 1.8	6.9 ± 1.5	
LH (IU/l)				
	1-28	5.3 ± 4.1	4.8 ± 3.4	
	Max	11.5 ± 7.5	8.8 ± 4.5	
E_2 (pmol/l)				
	1-28	303 ± 265	289 ± 199	
	Max	643 ± 394	538 ± 437	
P (nmol/l)				
	1-28	3.6 ± 6.5	2.2 ± 1.0	0.001
	Max	11.6 ± 15.7	3.1 ± 1.1	0.01
Follicle diameter (mm)				
	1-28	16.2 ± 8.7	16.1 ± 8.5	
	Max	24.8 ± 9.0	21.7 ± 9.9	

Mean and maximum P levels were significantly higher in the Org 31710 group (p=0.001 and 0.01 respectively; independent t-test). The distribution of all studied parameters was tested with the Mann Whitney U test and did not show any statistically significant differences. Follicle diameters and P serum values were categorised to allow for a classification based on contraceptive efficacy and displayed in Table II.

Table II

Distribution of maximum follicle diameters and maximum progesterone concentrations during a single, frequently monitored cycle (medication cycle 3-5) comparing ovarian activity during Org 31710 and placebo in users of 75 μg desogestrel progestagen-only contraceptive pills.

	Org 31710 (n=25)[a]		**Placebo** (n=23)	
	N	(%)	n	(%)
Follicle diameter [b]				
<10 mm	1	(4)	2	(9)
10-20 mm	4	(17)	9	(39)
21-30 mm	16	(67)	8	(35)
> 30 mm	3	(12)	4	(17)
Progesterone [c]				
< 10 nmol/l	18	(71)	23	(100)
≥ 10 nmol/l	7	(29)	0	

[a] 1 subject could not be classified in max. follicle diameter due to missing data.

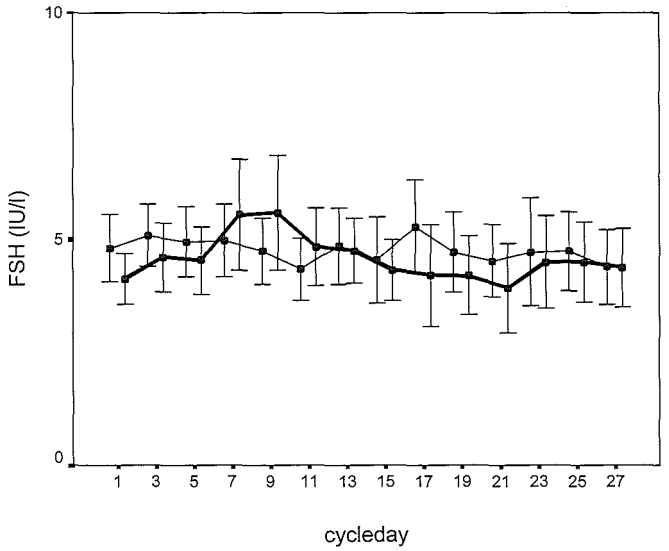
[b] distribution not statistically significant

[c] distribution statistically significant (p=0.005)

There were no statistically significant differences in the distribution of follicle categories between the two medication groups. However, the categories for P designed to discriminate for ovulation (Shepard and Senturia, 1977) indicated that ovulation was significantly more frequent in the Org 31710 group (p= 0.005; $\chi 2$ –test). In 6 out of 7 volunteers in which a serum progesterone > 10 nmol/l was found, sonographic signs for ovulation were present. In the remaining subject 1 ovary could not be visualised at the presumed moment of ovulation. Pituitary-ovarian parameters appeared normal in those women for whom ovulation was observed: LH peak 15.4 ± 10.1 IU/l; FSH peak 6.7 ± 1.1 IU/l; pre-ovulatory follicle diameter 21.0 ± 4.8 mm; E_2 peak 664 ± 237 pmol/l; maximum P levels were 26.0 ± 11.0 nmol/l (mean ± SD). Ovulation was mostly observed around mid-cycle i.e. in between monthly Org 31710 or placebo administration: day 8, 9, 10, 11, 12 (2 subjects, one without ultrasound conformation) and day 13. In 7 women (3

placebo, 4 Org 31710) sonographic suggestion for ovulation (sudden disappearance of a follicle > 20 mm) was observed without a progesterone rise in the next 7 days nor with a concomitant LH peak or pre-ovulatory E_2 levels.

In Figure 1 parameters of pituitary -ovarian activity are displayed during the monitored medication cycle of both groups.



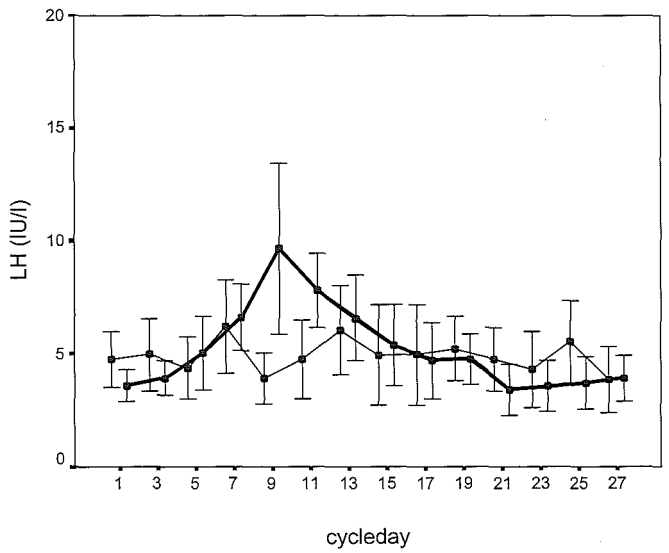


Figure 1

Pituitary-ovarian activity (mean ± 95% confidence interval) in 75 µg desogestrel + 150 mg Org 31710 group (▬▬, n = 25) versus 75 µg desogestrel +placebo group (——, n = 23). Each parameter is pooled for two consecutive cycle days (i.e. the first data set from cycle day 1&2, 3&4, etc.).

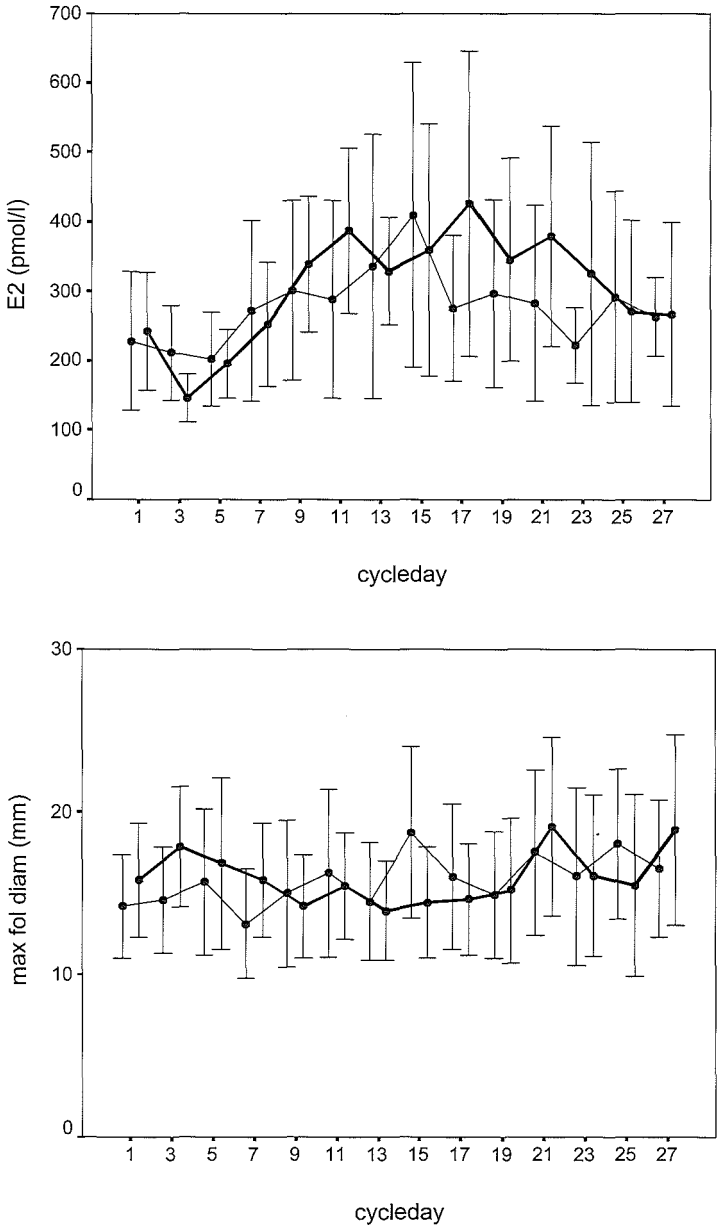


Figure 1 *(continued)*

Pituitary-ovarian activity (mean ± 95% confidence interval) in 75 µg desogestrel + 150 mg Org 31710 group (▬▬, n = 25) versus 75 µg desogestrel + placebo group (——, n = 23) .

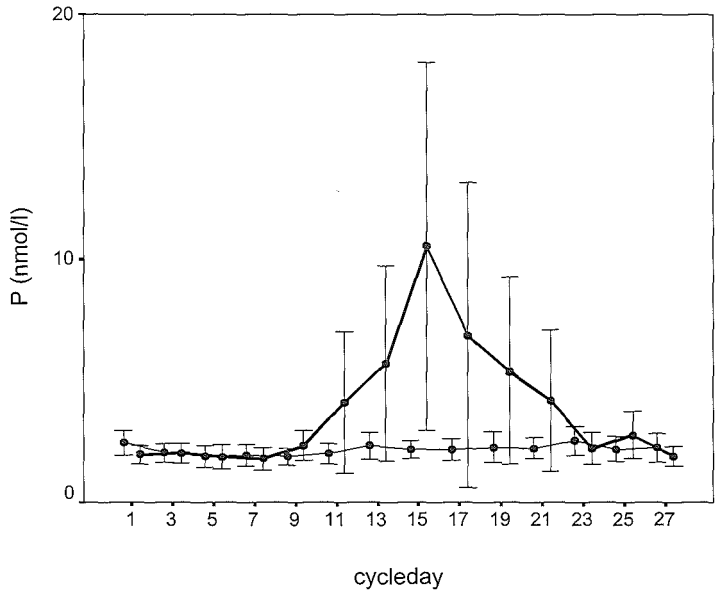


Figure 1 *(continued)*

Pituitary-ovarian activity (mean ± 95% confidence interval) in 75 µg desogestrel + 150 mg Org 31710 group (▬▬, n = 25) versus 75 µg desogestrel +placebo group (——, n = 23).

There were no statistically significant differences between the observations of the two centres. Since evaluation did not take place on a daily basis, each parameter is pooled for two consecutive cycle days (i.e. the first data set from cycle day 1&2, 3&4, etc.). LH levels were significantly higher in the Org 31710 group on day 9/10 ($p<0.001$) and 11/12 ($p=0.002$). Furthermore, E_2 levels were significant lower in the Org 31710 group on day 3/4 ($p=0.04$). Although mean P values were higher "mid-cycle" in the Org 31710 group due to the 7 observed ovulations, this did not reach statistical significance (Mann-Whitney U test). Since ovulation only occurred in the Org 31710 group, the ovulatory (n=7) and non-ovulatory (n=18) Org 31710 users were compared (data not shown). Significant differences were found on day 9 for follicle diameter ($p=0.05$) and E_2 concentration ($p=0.01$), on day 21 for LH concentration ($p=0.001$) and on day 13 through 21 for P ($p< 0.01$). In order to discriminate whether the differences between the Org 31710 group versus the placebo group were due to the occurrence of ovulations, the non-ovulatory volunteers using Org 31710 (n=18) were compared with the (non-ovulatory) placebo-group (n=23) (Figure 2).

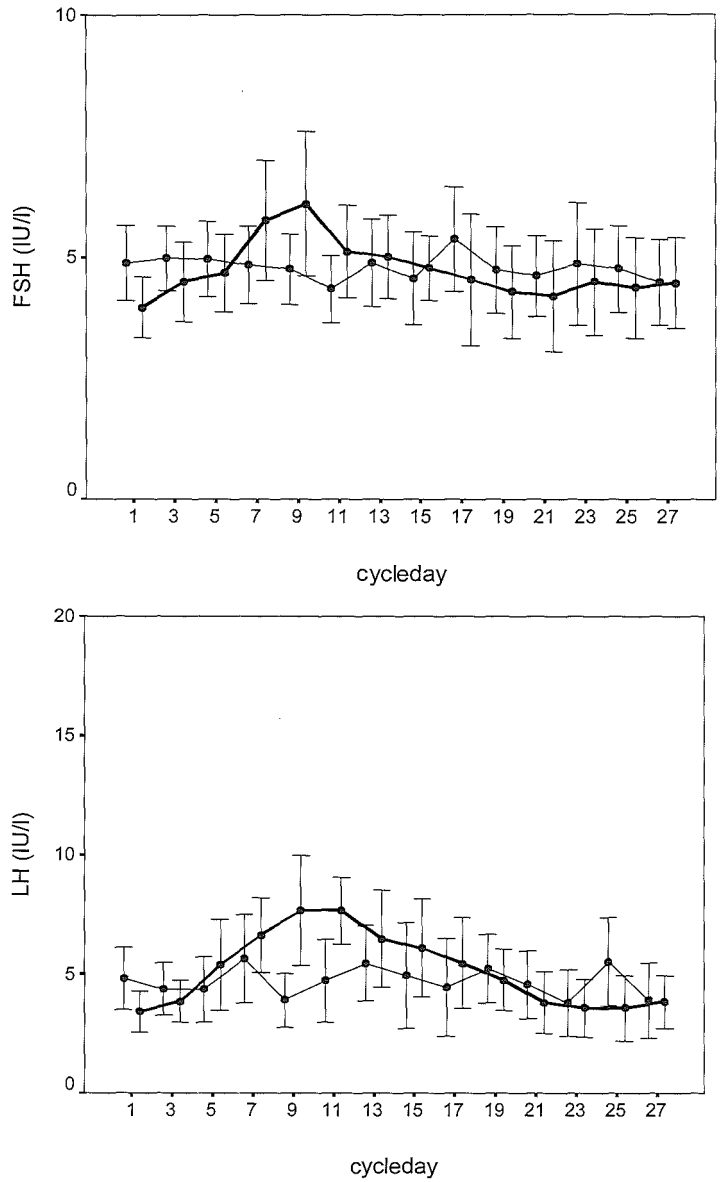


Figure 2

FSH and LH concentrations (mean ± 95% confidence interval) in non-ovulatory desogestrel + Org 31710 group (▬▬, n=18) versus desogestrel + placebo group(——, n=23). Each parameter is pooled for two consecutive cycle days (i.e. the first data set from cycle day 1&2, 3&4, etc.).

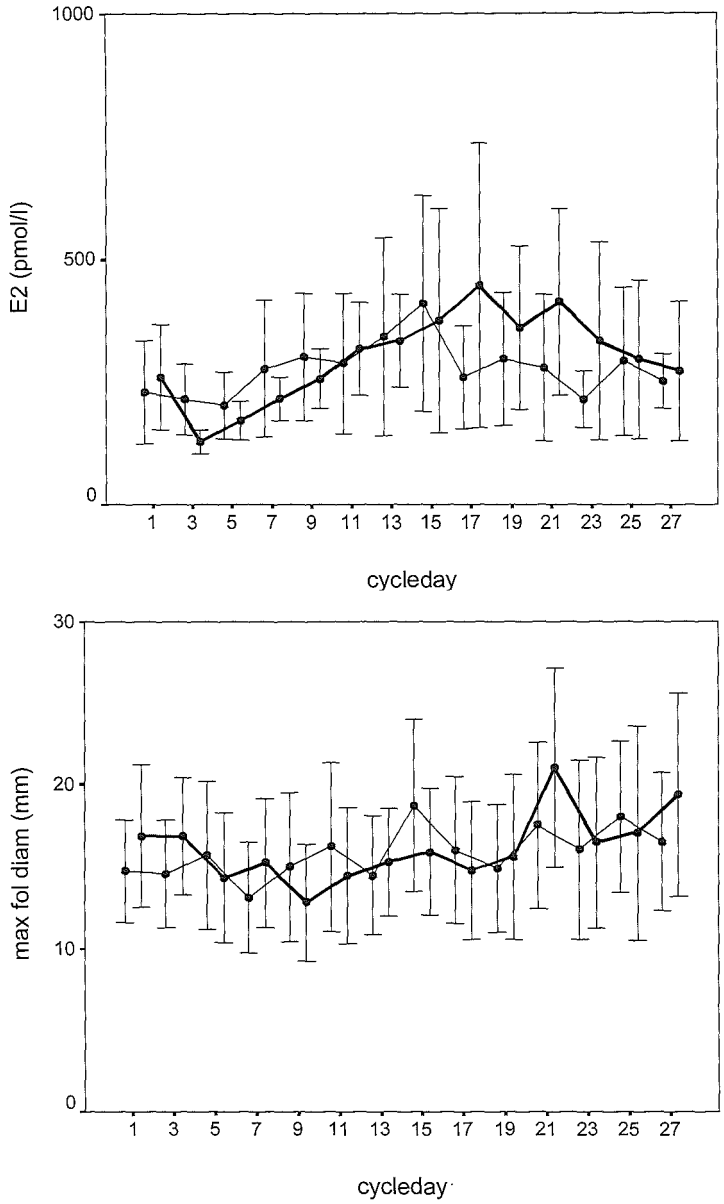


Figure 2 *(continued)*

Ovarian activity (mean ± 95% confidence interval) in non-ovulatory desogestrel + Org 31710 group (▬▬ , n=18) versus desogestrel + placebo group(——, n=23). Each parameter is pooled for two consecutive cycle days (i.e. the first data set from cycle day 1&2, 3&4, etc.).

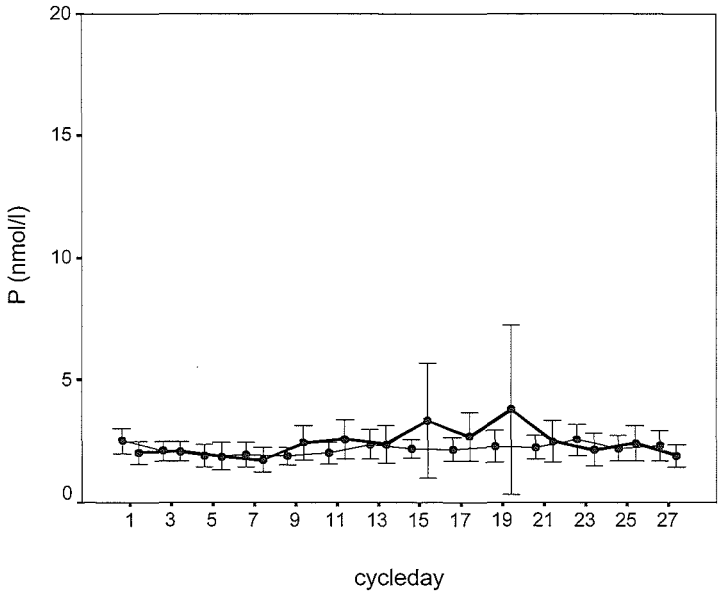


Figure 2 *(continued)*

Ovarian activity (mean ± 95% confidence interval) in non-ovulatory desogestrel + Org 31710 group (▬▬, n=18) versus desogestrel + placebo group (—— , n=23). Each parameter is pooled for two consecutive cycle days (i.e. the first data set from cycle day 1&2, 3&4, etc.).

E_2 levels remained significantly different on day 3 and LH levels on day 9 and 11 as was observed in the complete Org 31710 versus placebo groups. This indicates that the observed differences in E_2 and LH between Org 31710 and placebo are not only related to the occurrence of ovulation.

Endometrial thickness

The endometrium was significantly thicker in the Org 31710 group on cycle days 7 through 13 and cycle day 19 compared to the placebo group (Figure 3a). There was a weak but significant correlation between E_2 levels and endometrial thickness (Pearson's correlation coefficient: r =0.305, p=0.01 for the placebo group and r =0.317, p=0.01 for the Org 31710 group). The correlation was stronger when there was an ovulation during the study cycle (Pearson's correlation coefficient: r =0.302 versus r =0.42 ; p=0.01 comparing ovulatory versus non-ovulatory Org 31710 users (data not shown)).

In the Org 31710 group, no statistically significant differences were found in endometrial thickness in volunteers who did or did not ovulate (Figure 3b). Comparing all non-ovulatory volunteers, endometrial thickness showed again statistically significant differences on cycle day 7 through 13 and cycle day 19.

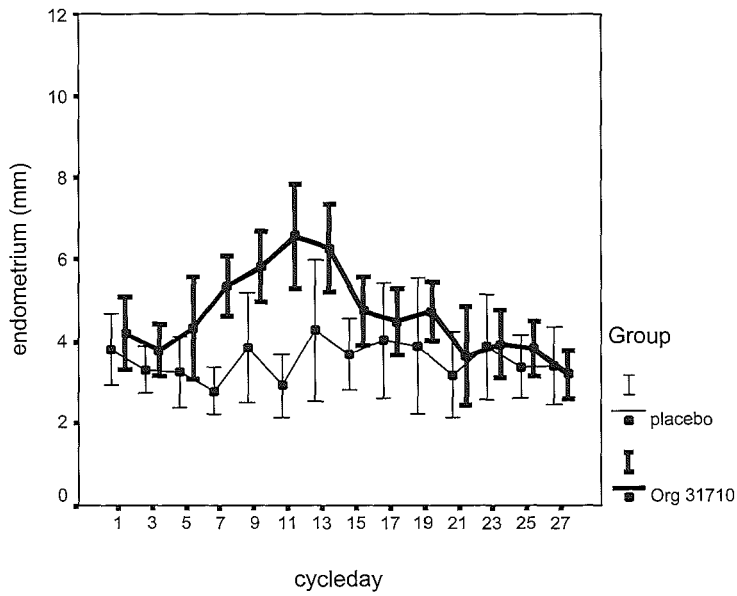


Figure 3

Endometrial thickness (mm, mean ± 95% confidence interval) measured by trans-vaginal ultrasound in desogestrel + Org 31710 versus desogestrel + placebo users.

Each parameter is pooled for two consecutive cycle days (i.e. the first data set from cycle day 1&2, 3&4, etc.).

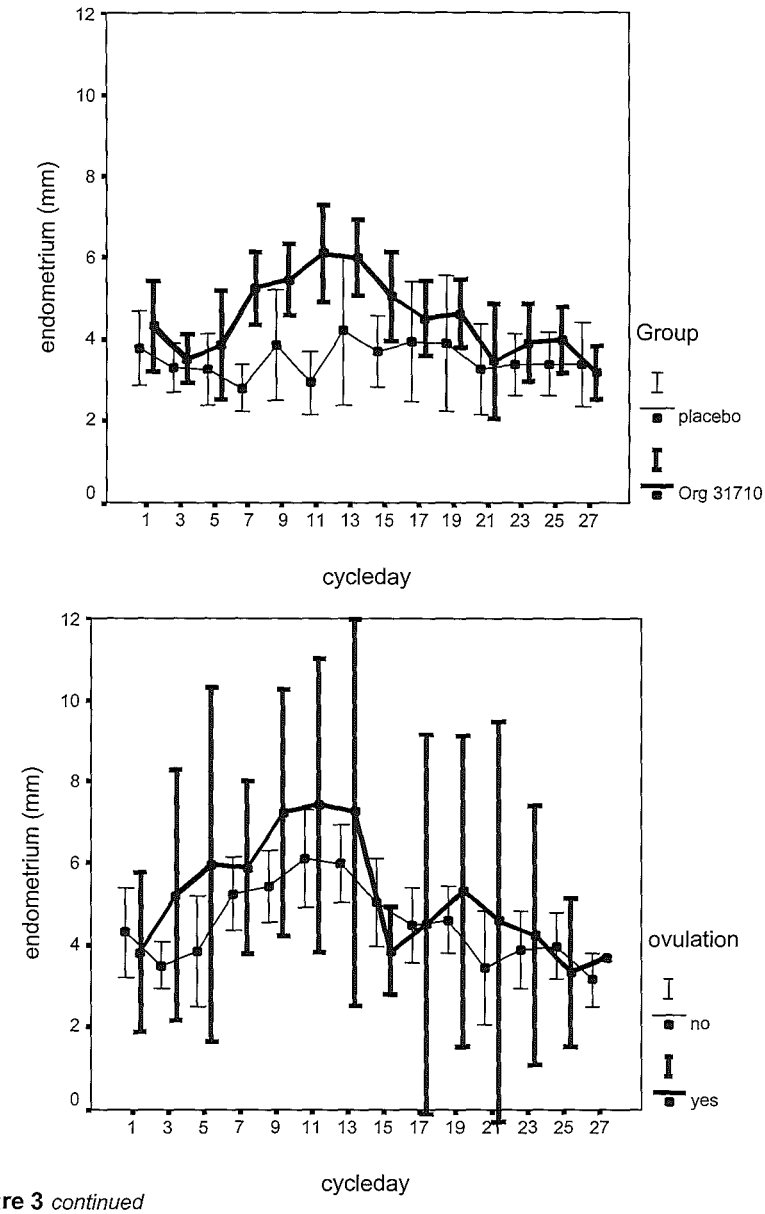


Figure 3 *continued*

Endometrial thickness (mm, mean ± 95% confidence interval) measured by trans-vaginal ultrasound in ovulatory versus non-ovulatory desogestrel + Org 31710 users (upper panel) and non-ovulatory desogestrel + Org 31710 vs desogestrel + placebo users (lower panel) Each parameter is pooled for two consecutive cycle days (i.e. the first data set from cycle day 1&2, 3&4, etc.).

4 Discussion

In this study we evaluated potential effects of a timed intermittent single dose of Org 31710 on pituitary-ovarian activity and endometrial thickness in healthy volunteers using 75 µg desogestrel progestagen-only contraception.

The contraceptive mode of action of progestagen-only-contraceptives is suppression of ovulation, suppression of midcycle peaks of LH and FSH and peripheral effects on cervical mucus, fallopian tube and endometrium (McCann and Potter, 1994). The effect of progestagen-only-contraceptives on the hypothalamic-pituitary-ovarian axis is incompletely documented. Progestagens exercise both negative and positive feedback actions on the hypothalamic pulse generator depending on dosage and chemical structure (Hemrika, 1993). Intra-ovarian regulation of folliculogenesis is also hypothesised to be affected through progesterone receptors in the theca layer (Hild-Petito *et al.*, 1988). Serum FSH levels seem to be less affected (Tafurt *et al.*, 1980), but generally peak levels of LH and FSH are diminished (Landgren *et al.*, 1979; Kim Bjorklund *et al.*, 1992). Ovarian activity ranges from complete suppression, dominant follicle formation without ovulation or luteal activity, ovulation with luteal insufficiency to normal ovulatory patterns (Landgren and Diczfalusy, 1980; Tayob *et al.*, 1986; Shaaban *et al.*, 1993). In progestagen-only-contraceptive users ovulatory cycles have been reported in up to 84% of women (McCann and Potter, 1994).

In this study, none of the 23 studied cycles in women using 75 µg DSG POP demonstrated an ovulatory pattern, while this occurred in 7/25 (28%) of cycles in the Org 31710 group. This finding is in agreement with an earlier study regarding pituitary-ovarian activity during the use of 75 µg DSG POP (Rice *et al.*, 1996). However, comparable levels of FSH, LH, follicle diameter and E_2 were observed in the Org 31710 and placebo group. Our data corroborate the observations that although gonadotrophin peak levels are suppressed, dominant follicles commonly emerge and moderate E_2 levels are present during POP therapy.

There were no statistically significant differences in overall (mean levels throughout a single cycle) or maximum levels of LH, FSH, E_2 and follicle diameter comparing Org 31710 users who did or did not ovulate. Furthermore, differences in sequential E_2 levels and follicle diameter only reached statistical significance on day 9 and for LH on day 21. Although there was a tendency for ovulation to occur at midcycle (i.e. in between Org31710 gifts), no parameter could be identified which could predict ovulation in the Org 31710 group.

Other attempts to combine an anti-progestagen and a progestagen for contraception

have been reported. A combination of mifepristone was given at a dose of 50 mg/day on cycle days 9-11 and 27-29 combined with medroxyprogesterone-acetate on cycle days 17-26 (Kekkonen *et al.*, 1995). Twenty of 32 cycles (63%) did not show evidence of ovulation (progesterone > 9 nmol/l) and serum E_2 concentrations were statistically significant higher after mifepristone administration.

Relatively small numbers in this study may prevent the detection of a causative correlation and mere coincidence could be responsible for the ovulations to occur only in the Org 31710 group. However, a possible explanation for the (timed) ovulations in the Org 31710 group could be hypothesised to occur as the result of two mechanisms. Firstly, Org 31710 could reduce the progestagen induced inhibition of pituitary-ovarian activity merely by competition of the anti-progestagen with the progestagen DSG. Alternatively, the anti-oestrogenic activity of Org 31710 could interfere with E_2 -mediated feed-back mechanisms resulting in increased follicular sensitivity to FSH and/or increased pituitary sensitivity to release LH. Previous studies have indicated that mifepristone can disrupt ovulation by inhibiting the positive feedback effect of oestrogens and, hence, prevent or delay the occurrence of a pre-ovulatory LH surge (Baird *et al.*, 1995). This effect may not occur at the level of the pituitary but rather at the hypothalamus (Heikinheimo *et al.*, 1996). Cyclic administration of mifepristone alone in different regimens showed partial inhibition of E_2 secretion and suppressed P levels (Spitz *et al.*, 1993). Daily administration of mifepristone in the guinea pig inhibited ovulation in a dose-dependent fashion (Batista *et al.*, 1991). In normally cycling women a dosage of 1 mg/ day delayed ovulation without affecting gonadal steroid production (Batista *et al.*, 1992). Baird and co-workers demonstrated that mifepristone 1 mg/day continuously was able to disrupt ovulation by inhibiting the positive feedback effect of oestrogens to produce the LH surge while basal levels of gonadotrophins remained unchanged (Baird *et al.*, 1995). Ovulation was delayed by approximately 7 days. These findings support the hypothesis that a direct effect of an anti-progestagen on hypothalamic-pituitary regulation could be responsible for the observation that ovulation clustered around midcycle in our study.

Probably the most important disadvantage of current progestagen-only-contraceptives is the unpredictable bleeding pattern. This bleeding pattern is often thought to develop as a result of both endogenous and exogenous endocrine influences on the endometrium. However, reports are conflicting. Some studies have failed to demonstrate a relationship between bleeding patterns and ovulation, ovarian hormones or exogenous progestagen levels (Kim Bjorklund *et al.*, 1991; Landgren *et al.*, 1994; Darney *et al.*, 1996) whereas others indicate that there might well be a relation between bleeding pattern and endogenous hormonal activity

(Zalanyi *et al.*, 1984; Shoupe *et al.*, 1991). The rationale of adding Org 31710 to a progestagen-only contraceptive regimen is derived from the concept that the addition of an anti-progestagen might improve cycle control. The anti-progestagen mifepristone was found to block oestrogen-induced endometrial proliferation in primates due to a non-competitive anti-estrogenic activity (Van Uem *et al.*, 1989). This action was found to be dose-dependent in the presence of physiological levels of E_2. Mifepristone was found to be antagonistic in the presence of P, but demonstrated endometrial progestational effects at low doses and an antiproliferative (anti-oestrogenic) effect at higher doses in the absence of P (Wolf *et al.*, 1989b). Bleeding patterns during the use of the studied medication are currently being analysed for all 104 subjects in which a more cyclic bleeding pattern is observed in the subjects of the Org 31710 treatment group. In the Org 31710 there was no correlation between the occurrence of ovulation and the bleeding pattern (reports will be reported seperately).

Continuous administration of mifepristone 1mg/day showed endometrial morphology similar to that seen in infertile women with luteal phase defects (Batista *et al.*, 1992). Onapristone, another anti-progestagen, also demonstrated an inhibitory effect on endometrium growth in primates (Ishwad *et al.*, 1993; Neulen *et al.*, 1996). Both an increase in the endometrial E_2 and P receptor concentrations (Neulen *et al.*, 1996) and the E_2 receptor of the endometrial stroma alone have been reported (Murphy *et al.*, 1995). In this study we have found a statistically significant increase of endometrial thickness following the use of Org 31710, irrespective of the occurrence of ovulation. This difference gained statistical significance on day 7, 9, 11, 13 and 19. Although continuous administration of mifepristone blocks oestrogen-induced endometrial proliferation, a single dose of Org 31710 in this study was associated with an increase in endometrial thickness. The significant increase of endometrial thickness compared with the placebo group was not associated with higher E_2 levels. A direct effect on the endometrium is therefore postulated, possibly through an increase in endometrial oestrogen receptors. Histological classification of the endometrium is mandatory to explore this further.

In conclusion; in this study we have assessed the influence of a single monthly dose of the anti-progestagen Org 31710 during a 75 µg DSG continuous regimen of POP. Our data suggest that Org 31710 may temporarily decrease E_2 production, presumably through a direct effect on ovarian function. Furthermore, it may disrupt E_2 related feed back mechanisms through an effect at the hypothalamic-pituitary level and delay the LH surge. This may increase the likelihood of ovulation to occur during the POP cycles studied. Finally, there appears to be a direct effect on endometrial proliferation. One should be cautious with comparing the effects of Org 31710 with known mifepristone effects since differences may be caused by different

mechanisms of action and/or different dosing schedules. Combining anti-progestagens with progestagen-only contraceptives could result in improved bleeding characteristics due to temporarily diminishing the effect of the progestagen and / or through the direct (proliferative) effect on endometrium. However, effects on the hypothalamic-pituitary-ovarian axis and peripheral contraceptive modalities of progestagen-only contraceptives following the use of anti-progestagens remain to be investigated.

Chapter 6

Improving cycle control in progestogen-only contraceptive pill users by intermittent treatment with a new antiprogestogen (Org 31710)

1 Introduction

Poor cycle control (Broome and Fotherby, 1990) is the most troublesome side effect of progestogen only oral contraception and contraceptive implants. Menstrual disturbance is by far the most common reason for discontinuation of these methods (Belsey, 1988; Glasier, 2002). However the avoidance of an oestrogen component has many advantages, in particular for those women at higher risk for venous thrombosis (Alving and Comp, 1992). In addition, for breast feeding women or women with complaints associated with the use of combined oral contraceptives, a progestogen-only pill (POP) may be the preparation of first choice.

Daily administration of 75 mcg desogestrel (Cerazette ®, Organon, Oss, The Netherlands) has been shown to be a safe and reliable contraceptive method with an efficacy comparable to low-dose combined oral contraceptives (Collaborative Study Group on the Desogestrel-containing Progestogen-only Pill, 1998). However, irregular bleeding remains a major clinical problem and satisfaction and long-term compliance may improve if these bleeding problems can be reduced.

Org 31710 is a synthetic 19-norsteroid possessing strong antiprogestogenic, low antiglucorticoid activity (Kloosterboer *et al.*, 1988) and weak androgenic/antiandrogenic activity (Kloosterboer *et al.*, 1994). If administered during the midluteal phase of the menstrual cycle at sufficiently high dosages, Org 31710 will, as other antiprogestogens, e.g. mifepristone, induce bleeding and shedding of the endometrium (Swahn *et al.*, 1988). Org 31710 appeared to be more potent than mifepristone for induction of menses in monkeys (Kloosterboer *et al.*, 1994). In an earlier study it was shown that a single dose of 150 mg Org 31710 was the lowest dose to induce a menstrual bleeding within 24-28 hours after ingestion

It has been demonstrated that the addition of an antiprogesterone to progesterone-only contraception reduces the incidence of unscheduled bleeding in monkeys (Williams *et al.*, 1994), and that the supplementary administration of an antiprogestogen to POP regimen may improve cycle control possibly as a result of blocking progesterone receptors in the endometrium (Hodgen *et al.*, 1994; Heikinheimo *et al.*, 1996). Once-a-month administration of mifepristone improves bleeding patterns in women using subdermal contraceptive implants releasing levonorgestrel (Norplant®) (Cheng *et al.*, 2000). The improvement in bleeding pattern could be either by a direct effect of antiprogestin on the endometrium as suggested by the effect on steroid receptor expression, or by inducing ovulation (Glasier *et al.*, 2002). An increased ovulation rate may jeopardize contraception, however no pregnancies occurred when

mifepristone was administered to Norplant users (Cheng *et al.*, 2000).

This study was designed to determine if the addition of the new antiprogestagen, Org 31710, at regular 28 day intervals to a POP regimen of 75 mcg desogestrel daily would improve cycle control in women using this form of contraception. As part of this study the effect on endocrine parameters was studied. These data have been published separately (Van Heusden *et al.*, 2000).

2 Subjects and Methods

Study design and medication

The study was performed in 6 centres in Edinburgh, Hull, Paris, Rotterdam, Santiago and Stockholm with ethic approval obtained from the supervisory body at each centre. The study was designed as a double-blind, placebo controlled study with a duration of minimally four and maximally seven consecutive treatment periods of 28 days.

Eligible subjects were given a continuous POP regimen consisting of 75 mcg of desogestrel daily and then randomised by computer code to receive in addition either a single dose of 150 mg (three tablets containing 50 mg each) of the antiprogestogen, Org 31710, or visually indistinguishable placebo tablets once every 28 days in a double-blind fashion. The first desogestrel and Org 31710/placebo tablets were both given on the first day of menstruation and therapy continued for between 16 to 28 weeks (4 to 7 cycles of 28 days). Therefore Org 31710/placebo tablets were given on a maximum of 7 occasions on days 1, 28, 56, 84, 112, 140 and 168.

Subjects

Women were eligible for participation in the study if they were healthy, between 18 and 45 years, had normal menstrual cycles with a mean length between 24 and 35 days (with an intra-individual variation of plus or minus 3 days) and a body mass index between 18 and 29 kg/m2. The menstrual history was asked for in detail at the Screening visit. The volunteers were advised to use barrier methods of contraception during the whole
treatment period unless they had undergone sterilization. Women who had taken recent steroid contraceptive therapy (orally one month, parental 6 months), who were lactating, had abnormal

haematological or biochemical values at screening, hypertension, PAP smear class III or higher and undiagnosed vaginal bleeding were excluded from participation.

Vaginal bleeding pattern

The occurrence of vaginal bleeding was documented on a daily basis on a diary card by the women themselves. Vaginal bleeding was indicated as spotting (requiring maximally one pad/tampon per day) or bleeding (requiring two or more pads/tampons per day). Analysis of bleeding patterns were performed, excluding women with major protocol violations, as a Per Protocol group, by cycle and reference period. Major protocol violations were defined as serious non-compliance with the study drugs, invalid data on the diary cards or deviations from inclusion/exclusion criteria. For the cycle analysis, treatment cycles were defined as the days between Org 31710/placebo intake. Based on the hypothesis that Org 31710 induces a regular bleeding pattern, each cycle was subdivided in two sections: a first section of the first seven days (Day 1-7: period of expected bleeding) and the other section (Day 8-28, when bleeding was not expected). The percentage of women with bleeding or spotting (B/S), the number of B/S days, and the occurrence of B/S episodes starting in a certain period of the cycle were calculated per woman, per cycle and per reference period.

Reference period analysis was performed as described by the World Health Organization using 90-day reference periods (Belsey *et al.*, 1986; Belsey and Farley, 1988; Rodriguez *et al.*, 1976). Bleeding was categorized in five bleeding pattern indices (amenorrhoea, infrequent bleeding, frequent bleeding, prolonged bleeding and irregular bleeding) following standard WHO definitions (Belsey *et al.*, 1986), with a modification for infrequent bleeding.

Amenorrhoea was defined as no B/S throughout a reference period; infrequent bleeding was defined as less than 3 B/S episodes starting within a reference period excluding amenorrhoea; frequent bleeding was defined as more than 5 B/S episodes starting within a reference period; prolonged bleeding was defined as at least one B/S episode starting within a reference period and lasting more than 14 days, and irregular bleeding was defined as a range of the length of bleeding-free intervals less than 17 days. Bleeding patterns were analysed for a shifted reference period (RP) which was defined as a period of 90 days, starting 28 days after the first day of treatment, i.e. Day 29-118. This period was chosen to exclude the bleeding episode at the start of the study, noted for all women since the study started on the first day of menses. This shifted reference period was considered more useful in comparing bleeding patterns between treatment groups.

Safety

Evaluation of safety was performed at screening, one to three days after each Org 31710/placebo intake and one to three days after the last desogestrel POP intake. Safety evaluation included assessment of haematology parameters, biochemistry and enzymes, urinalysis and vital signs. The numbers and nature of adverse events were recorded throughout the study period. For safety variables, only descriptive statistics were used.

Data analysis

For the cycle analysis, treatment cycles were defined as the treatment period starting on the Day of Org 31710/placebo intake and ending on the day before the next Org 31710/placebo intake. These cycles were subdivided into the first period of 7 days ("expected bleeding") and the remaining part of the cycle ("non-expected bleeding").

Variables (such as mean number of bleeding/spotting (B-S) days, occurrence of B-S episode) were calculated per subject, per cycle, and per period. Treatment group comparison was done using the Wilcoxon test stratified for centre. In addition, the variables were summarized for each subject across cycles 2-7. These intra-subject variables are presented and analysed using the Wilcoxon test, stratified for centre.

The reference period analysis as described by the World Health Organization using 90-day reference periods was done with inter-group comparison using the Wilcoxon test, stratified for centre. Differences between the treatment groups in the number of subjects who discontinued the study were analysed using the Cochran-Mantel-Haenszel test.

For safety parameters, descriptive statistics were used. Results were considered statistically significant if $P<0.05$.

3 Results

Subjects

One hundred-and-three women were randomised, 52 in the Org 31710 group and 51 in the placebo group. The Per Protocol group consisted of 102 women (51 women in each group). Three women (5.8%) in the Org 31710 group and nine women (17.6%) in the placebo group discontinued the study ($p=0.048$, Cochran-Mantel-Haenszel test). The most reported reasons for discontinuation were occurrence of unacceptable adverse events, "not willing to or cannot co-operate further" and bleeding irregularities. The mean treatment duration with 75 mcg

desogestrel POP was 161 days for the Org 31710 group and 152 days for the placebo group. Demographic data and menstrual bleeding characteristics at baseline were comparable for both groups (Table I).

Table 1

Demographic data and menstrual bleeding characteristics (mean and [range]) at baseline for all treated subjects.

	75 mcg DSG + Org 31710 (n=52)		**75 mcg DSG + placebo** (n=51)	
	Mean	*Range*	*Mean*	*Range*
Age (years)	31.6	20 - 44	32.8	22 – 45
Height (cm)	164.4	145.0 – 184.0	164.6	148.0 – 179.0
Weight (kg)	63.8	49.0 – 85.0	63.6	48.0 – 93.0
Body mass index (kg/m2)	23.6	19.0 – 29.0	23.5	18.0 – 29.0
Cycle length (days)	28.3	24 – 35	28.6	25 – 35
Usual duration of flow (days)	4.6	3 – 8	4.6	2 - 8

Bleeding pattern

The percentage of women who reported bleeding and/or spotting (B/S) is graphically presented per day in Figure 1. Since all women started treatment on the first day of the menses, a 100% B/S incidence is observed for both treatment groups on the first days of cycle 1. From cycle 2 onwards, the percentage of women with B/S per day in the placebo group was on average 30% during the whole treatment period and no days without reported B/S occurred. In contrast, a cyclic pattern was observed for the Org 31710 group; a peak incidence of B/S was observed on day 3 or 4 of each cycle, followed by a sharp decrease to 0% of the women on cycle days 9-15 and again a gradual increase in B/S incidence up to the next Org 31710 intake (Table II). When

only bleeding (no spotting) was taken into account, incidences were lower but followed the same pattern.

In the placebo group, two women (3.9%) as compared to none in the Org 31710 group discontinued because of an unacceptable bleeding pattern.

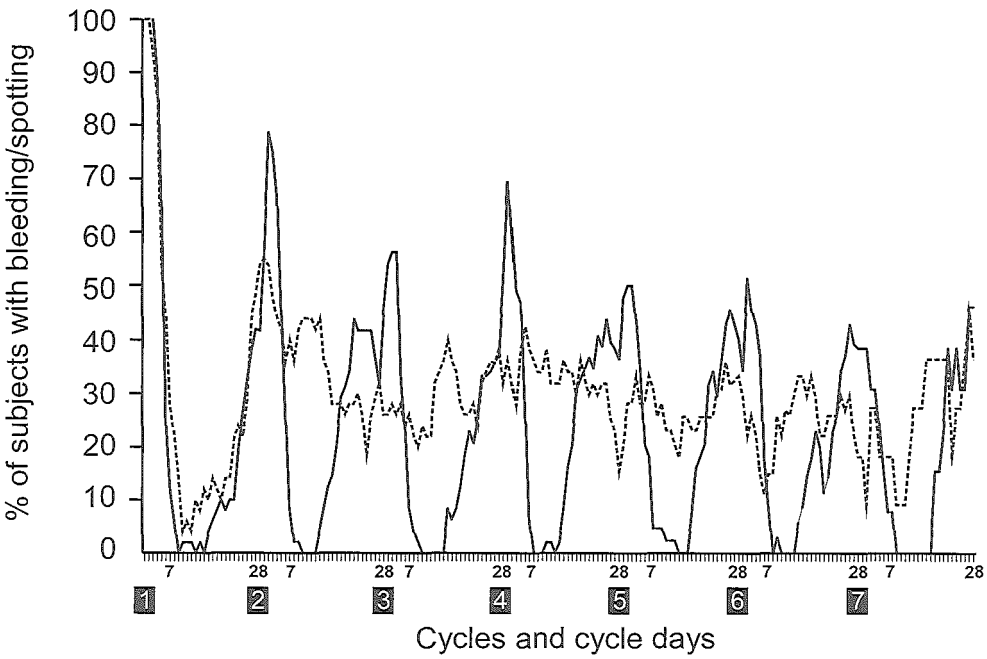


Figure 1

Percentage of subjects with bleeding-spotting in both treatment groups. Per Protocol Group.

\- - - - - - - : POP alone.

_____ : POP + 150 mg Org 31710 once every 28 days.

Table 2

Percentage of days with recorded bleeding and/or spotting (medians)

Cycle	75 µg DSG + Org 31710			75 µg DSG + Placebo		
	n	*Cycle day 1-7*	*Cycle day 8-28*	*n*	*Cycle day 1-7*	*Cycle day 8-28*
1	50	71.4	5.0*	50	71.4	12.5
3	48	42.9**	11.9***	50	0.0	23.8
6	35	28.6	18.2	27	0.0	14.3
2-7 comb[a]	51	45.7***	18.0***	50	28.6	29.6

Note: Cycles with major protocol violations were excluded from analysis.

*0.01< p ≤ 0.05; ** 0.001< p ≤ 0.01; *** p ≤ 0.001 (comparison between treatment groups)

[a] Within-subject summarization over cycles 2-7.

Cycle analysis

The median percentages of days with recorded B/S per cycle (and period of the cycle) are presented in Table 2 for cycle 1, 3, 6 and for cycles 2-7 combined. B/S on cycle days 1-7 (period of expected bleeding) was more frequent in the Org 31710 group compared with the placebo group. In the second period of the cycle (days 8-28), significantly less B/S was reported by the Org 31710 group compared to placebo in the first three cycles, whereas in cycles 4-7 no clear difference was observed. Similarly, more women reporting B/S episodes starting in, or with a part in cycle days 1-7 were observed in the Org 31710 group compared to the placebo group (Table III) except for cycle 7. The percentage of women reporting B/S episodes starting in the second period of a cycle (days 8-28) was comparable for both treatment groups.

Table 3

Percentage of subjects with a bleeding/spotting episode starting in or being part of the cycle periods.

	0.075 mg DSG + Org 31710			0.075 mg DSG + Placebo			
	Bleeding / Spotting episode						
Cycle	*n*	***starting*** *day 1-7*	***part of*** *day 1-7*	***starting*** *day 8-28*	*n*	***starting*** *day 1-7*	***part of*** *day1-7*	***starting*** *day 8-28*
1	50	100.0	100.0	52.0	50	100.0	100.0	62.0
3	48	52.1***	79.2***	58.3	50	26.0	48.0	72.0
6	35	34.3*	71.4*	77.1	27	11.1	40.7	59.3
2-7 comb[a]	51	49.9***	78.4***	69.8	50	28.8	55.0	62.5

Note: Cycles with major violations were excluded from analysis.

*0.01<p≤0.05; **0.001<p≤0.01; ***p≤0.001 (comparison between treatment groups)

[a] Within-subject summarization over cycles 2 – 7.

Reference period analysis

The reference period analysis divides the bleeding information into consecutive periods of 90 days (Table III). The treatment duration allows only the evaluation of the first reference period (day 1-90) and the reference period starting 28 days after the first treatment administration (presented as Ref. per #). Except for the number of B/S episodes, statistically significant differences between the two treatment groups were present for all the parameters, i.e., the number of B/S days, the mean length of B/S episodes (days) and the range of the length of bleeding fee intervals (days) were less for the Org 31710 group than for the placebo group. Although no significance was shown, the median number of BS episodes was smaller within the Org 31710 group than in the placebo group in both reference periods. The range of the length of

bleeding intervals is a measure for the variation in bleeding patterns. Amenorrhoea did not occur.

Table 4

Per Protocol Group, Reference Period Analysis

Variable	Reference Period[a]	**0.075 mg DSG + Org 31710**		**0.075 mg DSG + Placebo**		P-value
		n	*Median*	*n*	*Median*	
B/S days (n)	1	44	21.5	46	28.0	0.023
	2	41	23.0	38	31.0	0.007
B/S episodes (n)	1	44	3.0	46	4.0	0.31
	2	41	4.0	38	4.5	0.42
Mean length of B/S	1	44	5.0	46	6.5	0.031
episodes (days)	2	41	5.0	38	6.7	0.016
Range length of	1	44	14.5	46	22.5	0.001
B/free interval	2	41	19.0	38	22.5	0.023
(days)						

[a] Reference period 1 is from day 1-90; reference period 2 is from day 29-118.

Safety

Sixty-five percent (65%) of the subjects in the Org 31710 group and 59% of the subjects in the placebo group reported at least one adverse event. The most frequently reported were headache, emotional lability, acne and breast pain. Differences in incidence occurred mainly for acne (3.8% for Org 31710 and 11.8% for the placebo group) and breast pain (15.4% versus 5.9%). Three subjects (5.8%) in the Org 31710 group, two classified as possibly related and one unlikely to be related to the treatment, and four subjects (7.8%) in the placebo group, four probably related to treatment, discontinued due to unacceptable adverse events. These were headache, ovarian cyst, emotional lability, decreased libido, increased weight and hypertrichosis. For one of the women in the Org 31710 group it was regarded as a serious

adverse event (appendectomy).

No clinically relevant changes from baseline were observed for any of the measured haematology, biochemistry and urinalysis parameters. A difference between the Org 31710 and placebo groups was observed for body weight: 10 women in the Org 31710 group (19.2%) had an increase (6 women) or decrease (4 women) in body weight of more than 7% compared with one woman (2.0%) in the placebo group.

4 Discussion

The most common side effect and reason for discontinuation with progestogen-only contraception is bleeding disturbance (Broome and Fotherby, 1990). This may be caused by a direct effect of the progestogen on the endometrium and/or disturbed ovarian hormone production (Kim Bjorklund *et al.*, 1992; Lau *et al.*, 1996; Faundes *et al.*, 1998; Glasier, 2002). The present study represents the first pilot study investigating the effects of an antiprogestogen, Org 31710 on an oral progestogen-only regimen. A dose of 150 mg Org 31710 was selected based on its menses-inducing effect when administered during the midluteal phase in women with a regular menstrual cycle as observed in earlier studies. A single oral dose of 150 mg Org 31710 induced vaginal bleeding within 48 hours, whereas a dose of 75 mg Org 31710 demonstrated only a moderate antiprogestational effect as shown by the induction of menses in only 2 out of 6 women (Kloosterboer *et al.*, 1994).

In the present study we showed that the bleeding pattern of women using the 75 mcg desogestrel POP was significantly improved by the administration of 150 mg Org 31710 once every 28 days. A cyclic bleeding pattern was observed in the Org 31710 treatment group with a bleeding free period in all subjects and in all treatment cycles following the addition of Org 31710. The incidence of B/S was significantly higher during day 1-7 (period of expected bleeding) in the Org 31710 group compared with the control group, accompanied by a significantly lower incidence of B/S in the remaining part of the cycle (day 8-28, period of unscheduled bleeding). These differences were clearly observed in the initial treatment cycles, but were somewhat less pronounced during the later cycles of the treatment period. Thus cycle control in the Org 31710 group was not yet optimal, as also evidenced by the relatively high incidence of B/S episodes starting in the second period of the cycle. The seemingly better cycle control in the initial treatment cycles could reflect a decrease in effect over time but a more likely

explanation for this could be that by design, most of the subjects recorded bleeding data for only 4 cycles.

A similar improvement in bleeding pattern has been reported (Cheng *et al.*, 2000) in women using a levonorgestrel-releasing subdermal contraceptive implant in whom 50 mg mifepristone was given once every 4 weeks.

The mechanism behind the improvement in bleeding pattern is not known. As part of this study the effect of the treatment on pituitary-ovarian activity was also studied (Van Heusden *et al.*, 2000). The results further support the previously published finding that desogestrel 75 mcg daily in contrast to 30 mcg levonorgestrel daily (Collaborative Study Group on the Desogestrel-containing Progestogen- only Pill, 1998) completely inhibited ovulation. When Org 31710 was added to the desogestrel POP treatment increased serum progesterone levels (>10 nmol/l) indicating ovulation was found in 29% of the subjects. However, changes in serum FSH, E_2 and P levels could not predict ovulation and none of the measured parameters could be related to the observed bleeding pattern. Endometrial thickness assessed by transvaginal sonography was also greater on cycle days 7 – 13 and 19 in Org 31710 treated group (Van Heusden *et al.*, 2000). If sufficient doses of different antiprogestogens including Org 31710 are administered during the midluteal phase, endometriolysis and vaginal bleeding will occur (Kloosterboer *et al.*, 1994; Swahn *et al.*, 1988; Cameron *et al.*, 1996). There is little doubt that the bleeding is primarily due to an direct effect on the endometrium and not to a primarily effect on the Corpus Luteum. This is because the bleeding does not require a decrease in ovarian steroid secretion and it can be induced even when progesterone levels are artificially elevated by exogenous hCG administration(Croxatto *et al.*, 1985).

It has previously been demonstrated that in ovariectomized cynomolgus monkeys in the presence of progesterone, mifepristone is antagonistic but in its absence mifepristone exhibits endometrial progestational effects at low doses and an anti-proliferative (anti-oestrogenic) effect at higher doses (Wolf *et al.*, 1989a; Chwalisz *et al.*, 1991). Results indicate that the antiproliferative effect of the antiprogestogen is not due to a decrease in oestrogen receptor concentration as occurs during progesterone treatment. On the contrary, both oestrogen and progesterone receptor concentration increased significantly and to supra normal levels in ovariectomized monkeys on oestradiol replacement therapy when treated with mifepristone. A possible explanation is that the over expressed oestrogen receptor might not activate the post-receptor mechanism responsible for endometrial tissue growth (Neulen *et al.*, 1996). These effects support the suggestion that the effect of antiprogestogen on the bleeding pattern with desogstrel POP is mainly due to an effect on the endometrium and the

progesterone receptor (Heikinheimo *et al.*, 1996).

The contraceptive mode of action of the desogestrel POP depends on the suppression of ovulation and the midcycle peaks of LH, as well as on effects on cervical mucus, fallopian tube motility and endometrium (McCann and Potter, 1994). Since the addition of Org 31710 to the desogestrel treatment increases the rate of ovulation, a possible drawback may be an increased risk of contraceptive failure. However, the frequency of ovulation was similar to that observed with 30 mcg/day levonorgestrel (Collaborative Study Group on the Desogestrel-containing Progestogen- only Pill, 1998) and in the study by Cheng et al (Cheng et al., 2000), and yet no pregnancy occurred.

Interestingly the improved bleeding pattern in the study occurred in all women treated with the antiprogestogen. Although it is not known who ovulated and who did not, the 28 % ovulation rate might explain the improved bleeding pattern in that group, while the other anovulatory women also experienced an improved bleeding pattern. In light of an improved but not yet optimal bleeding pattern associated with this regimen and the possible decreased contraceptive efficacy with Org 31710 added to POP, further investigations will be necessary for the clinical development of an optimal Org 31710- POP regimen.

General discussion and conclusions

Residual ovarian activity during oral contraception is related to the extent of inhibitory effects of contraceptive compounds on endogenous pituitary gonadotropin release. Absence of inhibitory effects, e.g. during the pill-free period or following pill omissions, allows for a recovery of the hypothalamic-pituitary-ovarian axis. During the pill-free episode, restoration of events comparable with the early follicular phase of the normal menstrual cycle may even induce contraceptive failure. Recovery of pituitary-ovarian activity during the pill-free period is fairly well documented, but little is known with regard to properties of a COC which determine the magnitude of this recovery and which properties establish the ensuing control necessary for sufficient suppression in the beginning of the subsequent medication cycle.

In our first study, we compared 3 different low-dose oral contraceptives in order to evaluate the relative contribution of the ethinyl-estradiol dosage (20 mcg versus 30 mcg EE) and the type of progestin (desogestrel or gestodene) on the recovery of the pituitary-ovarian axis. It appeared that maximum follicle diameters were smaller in the 30 mcg EE group at the beginning of the pill-free period. The growth rate of the maximum follicle (increase in diameter of the leading follicle from day 1 to 7 of the pill-free period) was not different between the study groups. Thus, initial follicle diameters at the beginning of the pill-free period determine the probability of a dominant follicle to emerge. While FSH levels were not significantly different between the groups at the beginning of the pill-free period, FSH levels and FSH patterns during the pill-free period were markedly influenced by the absence or presence of dominant follicles. Thus the ethinyl-oestradiol dosage was of more importance that the type of progestin, despite the observation that inter-individual variations were sometimes larger than inter-group variations. It remains to be established if the ethinyl-estradiol dose-dependent suppression of FSH is the sole determinant of follicle diameter at the beginning of the pill-free period. In this respect, a study could be designed in which the ethinyl-estradiol medication is continued during the pill-free period.

At the beginning of a new medication cycle, the inhibitory effects of lower dosed contraceptive steroids are less effective in the initial suppression of FSH and control of follicular development. The lesser degree of suppression, both at the beginning of the pill-free period and at the start of the next medication strip, increase the likelihood of dominant follicles to emerge. In our second study, we established that FSH alone is sufficient for follicle growth during a high-dosed COC despite extremely low LH levels. A FSH-dose related increase in diameter of follicles was observed, whereas E2 levels were comparable between the study groups. Dominant follicles that emerged during FSH administration, continued to grow during the week in which the FSH administration was stopped and the COC was continued. In the group

receiving the lowest dose of FSH (75 IU recFSH), no dominant follicles were formed and in all groups the number of non-dominant follicles decreased following discontinuation of FSH. This suggests that inhibition of follicle development in COCs is mediated through FSH inhibition for non-dominant follicles while dominant follicles do not seem to be influenced by COCs. However, increase in diameter of a follicle-like structure as assessed by ultrasound does not necessarily indicate true optimal follicular maturation.

The development of dominant follicles during steroid contraceptive treatment should be regarded as an important and objective parameter in the assessment of ovarian suppression. Once dominant follicles emerge, decreasing FSH levels do not necessarily prevent further progress to pre-ovulatory follicles or even cysts. This indicates that a COC is unable to inhibit follicle growth through FSH suppression alone when dominant follicles are present. Suggestions for alternative medication regimen are presented to prevent dominant follicles during the pill-free period. Furthermore, a study design to benchmark different COCs in their ability to cope with dominant follicles is proposed. Such a benchmark should provide a basis for comparison between COCs based on objective parameters, provide insight in the often prominent inter-individual variations in suppression and recovery of the pituitary-ovarian activity. Moreover, practical guidelines regarding pill-ommissions could be established.

While it had already been established that dominant follicles remain their potential to ovulate despite the start of a new medication strip, it is unclear how long follicles remain responsive to a signal to induce ovulation. This 'functional lifespan' of a follicle may be relevant in case dominant follicles continue to grow and pill omission allows for a LH surge to occur. Based on our observations, dominant follicles present at the beginning of the pill-free period, are not associated with high E2 levels and do not respond to the changing gonadotropin levels during the pill-free period.

The combination of an antiprogestin during a fixed progestin-only medication schedule was developed to overcome the most troublesome side effect of the progestin-ony pill: poor cycle control. Daily administration of 75 mcg desogestrel has been shown to establish a reliable contraceptive method with a higher degree of ovulation suppression in comparison to progestins from earlier generations. While ovulation suppression appeared to be complete in all women using 75 mcg desogestrel alone, a single dose of an antiprogestin allowed ovulation to occur in 28% of the studied volunteers using a combination. No major differences were found in LH, FSH, E2 and follicle diameter between the women who use the 75 mcg desogestrel progestin-only pill alone or in combination with the anti-progestin. In fact, in those women using the combination, no parameter could be identified which could predict ovulation. The finding that

most ovulations occurred halfway between the antiprogestin gifts suggests that the antiprogestin could reduce the progestin induced inhibition of pituitary-ovarian activity merely by competition with the progestin desogestrel. Alternatively, the anti-estrogenic activity of the studied antiprogestin could interfere with E2-mediated feed-back mechanisms resulting in increased follicular sensitivity to FSH and/or increased pituitary sensitivity to release LH. This temporarily decrease in E2 production is established presumably through a direct effect on the ovary. The administration of a single dose of antiprogestin established a significant improvement of the bleeding patterns in users of 75 mcg desogestrel. Also, the mechanism behind this improvement remains speculative, while improvement was seen in women who ovulated and in women who did not. The finding that administration of an antiprogestin improves bleeding patterns, yet not establishing a predictable bleeding pattern, mandates further investigations in the benefit of combining progestins and anti-progestins.

Surveying the findings and conclusions in this thesis, recommendations can be made for future studies.

1 Prospective comparative studies testing the benchmark concept proposed in this thesis with regard to different compounds and different medication schedules in low dose oral contraception.

2 Studies evaluating the role of alternative paracrine/endocrine systems (e.g. inhibin / activin / follistatin) in predicting ovarian activity during oral contraceptive regimen.

3 Studies evaluating the functional life-span of dominant follicles regarding their potential to ovulate during oral contraceptive regimen.

4 Studies to develop alternative regimens improving suppression of pituitary-ovarian activity during the pill-free period in low-dose combined oral contraceptives.

5 Studies to develop regimens reducing the frequency and / or necessity of withdrawal bleeding episodes in combined oral contraceptives.

6 Studies to evaluate the influence of very low levels of LH on follicular development during recombinant FSH administration in ART.

7 Studies to evaluate the direct effect of progestins on folliculogenenis.

8 Studies to evaluate the clinical feasability of antiprogestin- POP regimen or even combining anti-progestins in continuous ultra-low dose COC regimen.

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SUMMARY

Chapter 1

This chapter provides a brief introduction to the background and study objectives presented in this thesis

Chapter 2

The focal point of this review is to summarize the effects of oral steroid contraceptives on residual pituitary-ovarian activity. The mode of action of progestins, oestrogens, anti-progestins and combinations of these compounds are described. The focus of this review concerns (pituitary-) ovarian activity in combined oral contraception, especially during the pill-free period, extention of the pill-free period and pill-omissions during normal use. A model is suggested comparing the pill-free period with the early follicular phase of a normal menstrual cycle. Finally, a study design is proposed for benchmarking combined oral contraceptives based on the ability of studied medications and/or new medication schedules to prevent dominant follicles.

Chapter 3

This study was performed to evaluate pituitary-ovarian recovery in the pill-free interval during use of three low-dose combined oral contraceptives (COC). Either the estrogen component or the progestin component was comparable in the study groups in order to evaluate their relative influence. Serum luteinizing hormone (LH), follicle-stimulating hormone (FSH) and estradiol (E_2) levels were measured and follicle number and size estimated by transvaginal sonography daily during the seven day pill-free interval in 44 healthy volunteers using three different low dose oral contraceptives. Healthy volunteers were enrolled using 20 μg ethinyl-estradiol (EE) + 75 μg gestodene (GSD, n= 15), 20 μg EE + 150 μg desogestrel (DSG, n=17) and 30 μg EE + 150 μg DSG (n= 12) given according to the usual regimen of daily one tablet during three weeks and one week pill-free interval. No ovulations were observed. Pituitary hormones were not statistically significantly different at the beginning of the pill-free interval between the study groups. FSH concentrations were significantly higher at the end of the pill-free interval in the 30 μg EE group compared to both 20 μg EE groups (7.0 (0.6-12.4) IU/l versus 4.9 (1.4-6.1) IU/l and 4.5 (2.4-7.4) IU/l; p=0.001). In both 20 μg EE groups a single persistent follicle (24 and 28 mm)

was present in one subject. Follicle diameters were statistically significantly smaller at the beginning and the end of the pill-free period in the 30 μg EE group compared to both 20 μg EE study groups. Dominant follicles (defined as follicle diameter ≥10 mm) were observed at the end of the pill-free interval in both 20 μg EE groups (in 27% and 18% of women, respectively) but not in the 30 μg EE group. Finally, the area-under-the-curve for E_2 was statistically significantly lower in the 30 μg EE group compared to both 20 μg EE groups. The EE content rather than the progestin component in the studied COCs determined the extent of residual ovarian activity at the beginning of the pill-free interval. Dominant follicles were only encountered in the 20 μg EE study groups.

Chapter 4

The objective of this study was to compare spontaneous recovery of pituitary-ovarian activity during the pill-free period following the correct use of low-dose oral contraceptives and subsequent ovarian function during the administration of exogenous recombinant FSH (recFSH) after switching to continued Lyndiol® (2.5 mg lynestrenol + 0.05 mg ethinyl-oestradiol) medication. A prospective, randomized, group-comparative, single-centre study was performed with the following schedule: Following the monitoring of the pill-free period (week 1) and subsequent treatment with Lyndiol® (for a total of 5 weeks), all subjects were randomly allocated to 1 of 4 groups receiving daily FSH injections for 1 week (75 IU, 150 IU, 225 IU recFSH or 150 IU purified urinary FSH (uFSH)) during the 4th week of Lyndiol® use. Thirty-six healthy volunteers aged 18-39 year, pre-study oral contraceptive use for at least 3 months, cycle length between 24-35 days, were enrolled. Serum FSH, LH and E_2 concentrations as well as transvaginal ultrasound assessment of the number and diameter of follicles > 2 mm were used to monitor pituitary ovarian function. At the start of the pill-free period following the pre-study contraceptive medication, 67% of the women presented with LH and FSH levels < 1 IU/l and only 1 follicle > 10 mm was observed. Initial levels of LH and FSH correlated ($P < 0.05$) with the extent of pituitary-ovarian activity during the pill-free period. At the end of the pill-free period a follicle > 10 mm had emerged in one subject only. During the first 3 days of Lyndiol® use eventually 7 women (19%) showed at least one follicle > 10 mm. During combined exogenous FSH and Lyndiol® administration, LH levels remained completely suppressed (< 0,5 IU/l) in all women studied. FSH levels as well as number and size of follicles increased with increasing doses of exogenous FSH in a dose-dependant manner. E_2 levels remained low in all

groups (< 150 pmol/l). During the week following FSH administration, FSH levels and E_2 levels decreased gradually while the number of follicles > 10 mm still increased. We have confirmed that dominant follicles > 10 mm are present at the end of the pill-free period and during the first days after resumption of pill intake. Once follicles > 10 mm arose at the end of the pill-free period, continued use of Lyndiol® did not reduce follicle diameters One week of Lyndiol® reduces pituitary-ovarian activity to levels observed after 3 weeks of low-dose pills. FSH administration during Lyndiol® resulted in dose-dependent follicle growth despite extremely low LH levels. E_2 secretion (56 $\pm$ 51 pmol/l) occurred to a limited and variable extent along with extremely low serum LH concentrations. Recovery of pituitary-ovarian activity at the end of the pill-free period is comparable to FSH levels and follicle dynamics following 7 days of 75-150 IU/l recFSH.

Chapter 5

Endocrine and ultrasound effects were studied of an intermittent (every 28 days), oral administration of 150 mg of the anti-progestagen Org 31710 during the continued daily use of 75 µg desogestrel (DSG) for progestagen-only contraception. A randomised, double-blind, placebo controlled two-centre study was conducted in 50 healthy volunteers. Serum LH, FSH, E_2 and P levels and follicle number and size as well as endometrial thickness was assessed by transvaginal sonography at least twice weekly during a single medication cycle (cycle 3 to 5). Forty-eight women were evaluated (Org 31710 n=25; placebo n=23). Seven ovulations were observed in the treated group versus none in the placebo group. LH levels were higher on day 9 and 11 and E_2 levels lower on day 3 in the treated group, irrespective to whether ovulation occurred. No parameter could predict ovulation. Endometrial thickness was greater on cycle days 7 through 13 and 19 in the treated group. However, within the Org 31710 group, no significant differences were found in volunteers who did or did not ovulate. Observed differences may be attributed to a competitive effect of Org 31710 with progestagen-induced suppression of the pituitary-ovarian axis, altered E_2 feedback mechanisms and/or altered receptor availability.

Chapter 6

The safety and efficacy of the antiprogestogen Org 31710 in improving cycle control in healthy women using the 75 mcg desogestrel progestogen-only pill was investigated. One hundred-and-

three women received either 150 mg Org 31710 or placebo once every 28 days, starting on day 1, for a duration of 4 to 7 treatment cycles. The percentage of women with bleeding or spotting (B/S) every day in the placebo group was on average 30% during the whole treatment period and no days without reported B/S occurred. In contrast, a cyclic pattern was observed for the Org 31710 group; a peak incidence of B/S was observed on day 3 or 4 of each cycle, followed by a sharp decrease to 0% of the women on cycle days 9-15. Compared to the control group, less subjects in the Org 31710 group reported infrequent, irregular, frequent or prolonged bleeding. These differences were clearly observed in the initial treatment cycles, but were somewhat less pronounced during the later cycles of the treatment period. A relatively high incidence of B/S episodes starting in the second section of the cycle was also observed.

SAMENVATTING

Hoofdstuk 1

Dit hoofdstuk geeft een korte introductie tot de achtergrond en vraagstellingen die in dit proefschrift aan de orde komen

Hoofdstuk 2

Het focus van dit overzicht is gericht op de effecten van orale anticonceptiva op resterende hypofyse-ovarium activiteit. Het werkingsmechanisme van progestagenen, oestrogenen, anti-progestagenen en combinaties van deze stiffen worden beschreven. Bijzondere aandacht krijgt de hypofyse-ovarium activiteit tijdens gebruik van orale anticonceptive, met name tijdens de pil-vrije periode, verlenging van de pil-vrije periode en de gevolgen van het vergeten van 1 of meerdere pillen tijdens normal gebruik. Een model wordt gepresenteerd waarbij de pil-vrije periode wordt vergeleken met de vroege folliculaire fase van de normale menstruele cyclus. Als laatste wordt een studiemodel voorgesteld om een gewogen vergelijking mogelijk te maken tussen verschillende orale anticonceptiva gebaseerd op het vermogen om het ontstaan van dominante follikels te verhinderen.

Hoofdstuk 3

Deze studie werd uitgevoerd om het herstel te evalueren van de hypofyse-ovarium activiteit tijdens de pil-vrije periode van 3 laaggedoseerde orale anticonceptiva (OAC). Van deze OAC's was of de oestrogene component of de progestagen component onderling vergelijkbaar zodat de relatieve invloed hiervan beoordeeld kon worden. Serum Luteiniserend Hormoon (LH), Follikel Stimulerend Hormoon (FSH), oestradiol (E_2) werden gemeten alsmede follikelgrootte en follikelaantal middels vaginale echoscopie op dagelijkse basis tijdens de 7 pil-vrije dagen in 44 gezonde vrijwilligsters. Van hen gebruikten 15 een combinatie van 20 mcg ethinyl-estradiol (EE) + 75 mcg gestodene (GSD), 17 een combinatie van 20 mcg EE + 150 mcg desogestrel (DSG) en 12 een combinatie van 30 mcg EE + 150 mcg DSG volgens het gebruikelijke voorschrift van 1 pil per dag gedurende 3 weken gevolgd door een pil-vrije periode van 7 dagen. Er werden geen ovulaties waargenomen. FSH en LH waren niet statistisch significant verschillend in het begin van de pil-vrije periode tussen de groepen. FSH concentraties waren significant

verschillend aan het einde van de pil-vrije periode in de 30 mcg EE groep in vergelijking tot de 20 mcg EE groepen (7.0 (0.6-12.4) IU/l versus 4.9 (1.4-6.1) IU/l en 4.5 (2.4-7.4) IU/l; p=0.001). In beide 20 mcg EE groepen was een persisterende follikel (24 en 28 mm) aanwezig in 1 vrijwilligster. Follikeldiameters waren statistisch significant kleiner in het begin en aan het einde van de pil-vrije periode in de 30 mcg EE groep in vergelijking tot de 20 mcg EE groepen. Dominante follikels (gedefinieerd al seen follikel diameter $\geq$ 10 mm) werden waargenomen aan het einde van de pil-vrije periode in beide 20 mcg EE groepen (18% en 27%), doch niet in de 30 mcg EE groep. De 'area-under-the-curve' voor E_2 was statistisch significant kleiner in vergelijking met beide 20 mcg EE groepen.

De oestrogene component bepaalde meer dan de progestagene component de mate van resterende ovariumactiviteit in de pil-vrije periode van de bestudeerde OAC's . Dominante follikels werden enkel geobserveerd in de 20 mcg EE groepen.

Hoofdstuk 4

Het doel van deze studie was het vergelijken van het spontane herstel van hypofyse-ovarium activiteit tijdens de pil-vrije periode van laaggedosseerde OAC's en de ovarium activiteit gedurende de toediening van recombinant FSH (recFSH) tijdens het gebruik van een hooggedosseerde OAC (Lyndiol® (2.5 mg lynestrenol + 50 mcg EE). Een prospectieve en gerandomiseerde studie werd uitgevoerd waarbij het volgende studieschema van toepassing was: Na evaluatie van de pil-vrije periode (week 1) kregen alle vrijwilligsters gedurende 5 weken Lyndiol®. Alle vrijwilligsters werden gerandomiseerd in 1 van 4 groepen : dagelijkse injectie met FSH (75 IU, 150 IU, 225 IU recFSH of 150 IU gezuiverd urinair FSH (uFSH) gedurende de 4e week Lyndiol® gebruik. Zesendertig gezonde vrijwilligsters tussen de 18 en 39 jaar werden geincludeerd in deze studie waarbij het gebruik van OAC's tot 3 maanden voor de studie niet was toegestaan en de cycluslengte tussen de 24 en 35 dagen bedroeg. Serum FSH, LH en E_2 concentraties en bepaling van follikelgrootte en follikelaantal middels vaginale echoscopie werden bepaald als maat voor hypofyse-ovarium activiteit. Bij aanvang van de pil-vrije periode van de laaggedoseerde OAC's die voor de studie werden gebruikt werden bij 67% van de vrijwilligsters een LH en FSH < 1 IU/l en slechts 1 dominante follikel gezien. De concentraties voor LH en FSH aan het begin vn de pil-vrije periode correleerde statistisch significant (P< 0.05) met de mate van hypofyse-ovarium activiteit gedurende de pil-vrije periode. Aan het einde van de pil-vrije periode werd wederom slechts 1 dominante follikel waargenomen, doch gedurende de eerste 3 dagen van Lyndiol® gebruik werd dit bij uiteindelijk 7 vrouwen (19%) gezien. Gedurende de gelijktijdige toediening van FSH tijdens Lyndiol gebruik, bleven LH

concentraties volledig onderdrukt (< 0.5 IU/l) in alle vrijwilligsters. Zowel FSH als aantal en grootte van follikels nam toe tijdens FSH toediening in een mate die dosis afhankelijk was. E_2 concentraties bleven eveneens laag: < 150 pmol/l. Tijdens de week na de FSH toediening daalden FSH en E_2 concentraties geleidelijk terwijl het aantal dominante follikels nog toenam.

In deze studie bevestigen we het voorkomen van dominante follikels in de pil-vrije periode en gedurende de eerste dagen van pilgebruik. Indien dominante follikels ontstonden werd geen onderdrukking gezien door Lyndiol® gebruik. Na 1 week van Lyndiol® gebruik werd een vergelijkbare mate van hypofyse-ovarium activiteit gezien als na 3 weken laaggedoseerde OAC's. FSH toediening tijdens Lyndiol® gebruik resuteerde in een dosisafhankelijke follikelgroei ondanks extreme lage LH waarden. E_2 productie (56 $\pm$ 51 pmol/l) was beperkt en variable tijdens deze lage LH warden. Herstel van hypofyse-ovarium activiteit aan het einde van de pil-vrije periode is vergelijkbaar met FSH concentraties en follikelkarakteristieken volgend op 7 dagen75-150 IU/l recFSH.

Hoofdstuk 5

In deze studie werd de 4 weekselijke toediening van een oral anti-progestageen Org 31710 onderzocht tijdens het continu gebruik van 75 mcg DSG als progestageen-alleen anticonceptivum. Een gerandomiseerde, dubbel-blinde, placebo-gecontroleerde studie werd uitgevoerd in 2 centra bij 50 gezonde vrijwilligsters. Serum LH, FSH, E_2 en progesteron (P) alsmede echoscopische beoordeling van follikelgroei en endometriumdikte werden ten minste 2 maal per week gemeten tijdens een medicatie cyclus van 28 dagen (cyclus 3,4 of 5). Achtenveertig vrijwilligsters konden worden geevalueerd (Org 31710 n=25; placebo n=23). Zeven ovulaties warden waargenomen in de studiegroep en geen enkele in de placebogroep. LH concentraties waren hoger op dag 9 en 11 en E_2 waren lager op dag 3 in de groep die Org 31710 kregen, onafhankelijk of er nu wel of niet ovulatie optrad. Geen parameter kon worden geidentificeerd die ovulatie kon voorspellen. Het endometrium was het dikker op cyclusdag 7-13 en 19 in de behandelde groep. De waargenomen verschillen kunnen worden toegeschreven aan een competatief effect van Org 31710 met de door progestagenen geinduceerde onderdrukking van hypofyse-ovarium activiteit, een veranderd E_2 terugkoppelingsmechanisme en/of een veranderde receptorstatus.

Hoofdstuk 6

De veiligheid en werkzaamheid van het anti-progestageen Org 31710 tijdens 75 mcg DSG in

verbetering van bloedingsspatroon werd onderzocht in deze studie.Honderddrie gezonde vrijwilligsters kregen 4-wekelijks hetzij 150 mg Org 31710 hetzij een placebo op dag 1 gedurende 4-7 cycli. Het percentage vrouwen met bloedverlies of spotting (B/S) elke dag in de placebogroep was gemiddeld 30 % gedurende de gehele studieperiode en geen dagen zonder B/S werden gerapporteerd. In de Org 31710 groep daarentegen, werd een cyclisch bloedingspatroon gezien met een voorkeur voor dag 3 of 4 van iedere cyclus en een scherpe daling tot afwezigheid van B/S op cyclusdagen 9-15. Vergeleken met de controle groep werd in de Org 31710 groep minder vaak irregulair, infrequent, frequent of aanhoudend bloedverlies gerapporteerd. Deze verschillen waren met name duidelijk in de eerste cycli, doch wat minder duidelijk in de latere cycli van behandeling. Een relatief hoge incidentie van B/S episoden in de tweede helft van de cyclus werd eveneens waargenomen.

PUBLICATIONS

Van Heusden,A.M. and Fauser,B.C.J.M. (1996) FSH and ovarian function during combined oral contraceptive regimens. In Fauser,B.C.J.M. (ed), FSH action and intraovarian regulation. The Parthenon Publishing Group, London, pp. 211-221.

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CURRICULUM VITAE AUCTORIS

Arne Marius van Heusden werd op 21 mei 1960 geboren in Bilthoven. Hij groeide op in Dordrecht alwaar aan het Titus Brandsma College het VWO-B diploma werd gehaald in 1978. Na uitloting gaf een jaar Scheikunde aan de Rijksuniversiteit te Utrecht een goed inzicht in de sociale aspecten van het studentenleven. De studie Geneeskunde werd gevolgd aan de Rijksuniversiteit te Utrecht tussen 1979 en 1986. Aansluitend was hij arts-assistent in het Maria Ziekenhuis (thans Tweesteden Ziekenhuis) te Tilburg (opleider Dr. H.C.L.V. Kock). Na een kort verblijf in het St. Elisabeth Ziekenhuis (opleider Dr.J. de Graaf) werd hij arts-assistent in het Zuiderziekenhuis (thans Medisch Centrum Rijnmond-Zuid, locatie Zuider) te Rotterdam (opleider Dr. M. van Lent) tussen 1988 en 1990. Het uitblijven van opleidingsmogelijkheden in Rotterdam bood de mogelijkheid tot het doen van wetenschappelijk onderzoek leidend naar een dissertatie. Hierbij viel de keus uiteindelijk op een grote multicenter studie naar de betrouwbaarheid, bijwerkingen en bloedingpatronen van een combinatie van 75 mg melatonine en 500 mcg norethisterone als anticonceptivum (Prof. Dr. A.C. Drogendijk e.a.). In 1993 werd dit onderzoek voortijdig gestaakt wegens teleurstellende resultaten. Publicatie van de onderzoekresultaten was niet mogelijk. Aansluitend (september, 1993) werd gestart met het onderzoek dat ten grondslag ligt aan dit proefschrift onder leiding van Prof. Dr. B.C.J.M. Fauser (Sector Voortplantingsgeneeskunde, Afd. Gynaecologie-Verloskunde, Academisch Ziekenhuis 'Dijkzigt'). Tevens werd een aanvang gemaakt met de opleiding tot gynaecoloog in het Zuiderziekenhuis (december, 1993) en het Academisch Ziekenhuis 'Dijkzigt' (thans Erasmus Medisch Centrum) te Rotterdam (opleiders Prof. A.C. Drogendijk, Prof. Dr. H.C.S. Wallenburg en Prof. Dr. Th. J.M. Helmerhorst). Als laatst opgeleide Rotterdamse gynaecoloog van het vorige millennium werd op 1 december 1999 de opleiding afgesloten. Na een kort doch plezierig verblijf in Limburg (Maaslandziekenhuis te Sittard) is hij sedert september 2000 werkzaam als gynaecoloog in het MCRZ locatie Zuider te Rotterdam.

DANKWOORD

Blessed is he who expects no gratitude, for he shall not be disappointed.
W.C. Bennett

In den beginne waren er Hans Kock, Piet-Hein van Kessel, Hans Merkus en Piet van Vugt. Ik ben hen zeer erkentelijk voor de wijze waarop zij mijn jeugdig enthousiasme ruimte gaven en mijn keuze voor het vak grondvesten verleenden. Daarnaast is het volledig de 'schuld' van Hans Merkus dat ik plezier heb gevonden in het doen van onderzoek.

Prof.dr. B.C.J.M. Fauser, beste Bart , er is tenminste 1 voordeel aan een lange promotieperiode: je maakt samen nog eens wat mee. Na het melatonine-avontuur ben ik als wees in je groep opgenomen en heb me sindsdien gesteund gevoeld door je professionele en persoonlijke betrokkenheid. De combinatie van goede onderzoeksomstandigheden, wetenschappelijke uitstapjes en de nodige aandacht voor de sociale aspecten binnen de groep van 'jouw' onderzoekers maken het een groot plezier om terug te kijken. Je kritische blik, streven naar 'focus' en 'high standards', motivatie en enig geduld hebben hun vruchten afgeworpen. Dank^2.

Prof.dr. H.J.T. Coelingh Bennink, Prof.dr. F.H. de Jong en Prof.dr. J.W. Wladimiroff dank ik voor het kritisch doorlezen van het manuscript.

Bijzonder dankbaar ben ik geweest met de vrijwilligsters die participeerden in de klinische studies. Daar waar de belasting van bloedafname en echo hoog was, 6 uur 's ochtends voor menigeen toch wel erg vroeg was, bleek inzet en doorzettingsvermogen boven ieders verwachting. Dit gold ook voor de medewerkers van de Clinical Research Unit van het Academisch Ziekenhuis die een gezellige basis en de nodige ondersteuning boden voor de uitvoer van het onderzoek. Mijn collegae arts-assistenten in het Zuiderziekenhuis ben ik dankbaar voor de gestolen uurtjes in ochtend en avond die het mogelijk maakten om elke dag 2 sessies in het Dijkzigt door te brengen en toch nog 8 uur per dag te slapen. Jullie bewezen eens te meer dat collegialiteit omgekeerd evenredig is met het aantal mensen die dat moeten opbrengen.

De 'follikel-boys' Thiery Pache, Dick Schoot, Evert van Santbrink, Bernd Berning, Jits Schipper, Babek Imani, Diederik de Jong en de 'eiblaasjes-dames' Femke Hohman, Annemarie de Vet, Nicole Beckers en Annemarie Mulders vormden een uitermate plezierige groep lotgenoten die steun en gezelligheden boden als 'challenging material' voor de 'deadline' moest worden 'gefocust'.

Mijn maten Mat van Lent, Arie Verhoeff, Gemma Ras en Adriaan Logmans ben ik niet alleen dankbaar voor hun gastvrije inborst om mij zelfs 4 keer toe te laten in hun midden, maar ook voor de tijd die mij vergund werd om te werken aan onderzoek en het schrijven van dit proefschrift.

Mijn paranimfen Bram Preesman en Jan Roest ben ik zeer erkentelijk voor hun steun bij de plechtigheid, doch bovenal voor hun vriendschap. Weledelzeergeleerde P, Utrechtse maat en eiland van rust in een turbulente tijd en Weledelgeleerde Roest, Rotterdamse maat en Brother in Arms, het is een genoegen deze keer de rollen om te draaien.

Mijn diepste dankbaarheid gaat uit naar mijn ouders die ruim 40 jaar hun niet aflatende steun en liefde hebben geschonken en naar Inge en Hugo voor het verdragen van mijn houding jegens 'gezelligheid', het nachtelijk gestommel in mijn werkkamer en de vierkante ogen de dag erna.