

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

A.M.van Heusden¹, S.R.Killick²,
H.J.T.Coelingh Bennink³ and B.C.J.M.Fauser^{1,4}

¹Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Erasmus University Medical Centre Rotterdam, 3015 GD Rotterdam, The Netherlands; ²Department of Obstetrics and Gynaecology, University of Hull, Hull HU8 9HE, UK and ³Medical Research and Development Unit, N.V. Organon, 5340 BH Oss, The Netherlands

⁴To whom correspondence should be addressed

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 randomized, double-blind, placebo-controlled two-centre study was conducted in 50 healthy volunteers. Serum luteinizing hormone (LH), follicle stimulating hormone (FSH), oestradiol and progesterone concentrations, and follicle number and size were studied, as well as endometrial thickness, which was assessed by transvaginal sonography at least twice weekly during a single medication cycle (cycle 3–5). 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 concentrations were higher on days 9 and 11 and oestradiol concentrations lower on day 3 in the treated group, irrespective of whether ovulation occurred. No parameter could predict ovulation. Endometrial thickness was greater on cycle days 7–13 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 oestradiol feedback mechanisms, and/or altered receptor availability.

Key words: anti-progestagen/contraception/endometrium/pituitary–ovarian activity/progestagen only contraception

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 intrauterine devices, 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 (Van Uem *et al.*, 1989) demonstrated in monkeys that the anti-progesterone mifepristone could induce amenorrhoea by inhibiting oestrogen-induced endometrial proliferation. 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.*, 1989). In the presence of oestradiol both mifepristone and onapristone were able to increase the number of endometrial oestradiol and progesterone 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.

Material 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 concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), oestradiol and progesterone, 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 multicentre study ($n = 104$; six 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 2 months after a delivery or abortion, abnormal haematological or biochemical values at screening, Papanicolaou 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 (N.V.Organon, Oss, The Netherlands) was given as oral tablets of 75 µg for 196 days (seven cycles of 28 days). Org 31710 (N.V.Organon) 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 three 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 at 2000 g for 15 min within 2 h of withdrawal. Serum oestradiol concentrations were measured by radioimmunoassay (Diagnostics Products Corporation; Los Angeles, CA, USA). Progesterone concentrations were determined by radioimmunoassay as previously described (De Jong *et al.*, 1974). Serum FSH and LH concentrations were determined by immunoradiometric assay (Delfia kits, Kabi Pharmacia, Türkü, Finland). Intra-assay and inter-assay coefficients of variation (CV) were <4.0 and <6.4% for FSH, <15.5, and <14.1% for LH, <15 and <18% for oestradiol, and <16 and <17% for progesterone 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 $P < 0.05$.

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 ± 6.0 versus 29.1 ± 5.4 years, weight 65.5 ± 9.5 versus 65.6 ± 10.2 kg, height 167.3 ± 7.8 versus 168.0 ± 5.3 cm; Org 31710 ($n = 25$) versus placebo ($n = 23$), mean ± SD].

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

	Org 31710 ($n = 25$)	Placebo ($n = 23$)	<i>P</i> 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	
Oestradiol (pmol/l)			
1–28	303 ± 265	289 ± 199	
Max	643 ± 394	538 ± 437	
Progesterone (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	

FSH = follicle stimulating hormone; LH = luteinizing hormone.

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

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

^aOne subject could not be classified in maximum follicle diameter due to missing data.

^bDistribution not statistically significant.

^cDistribution statistically significant ($P = 0.005$).

Pituitary–ovarian activity

Forty-eight women were included in the evaluation of pituitary activity. Summary statistics for FSH, LH, oestradiol, progesterone, and follicle diameter are presented in Table I. Mean and maximum progesterone concentrations 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 progesterone serum values were categorized to allow for a classification based on contraceptive efficacy and are displayed in Table II. There were no statistically significant differences in the distribution of follicle categories between the two medication groups. However, the categories for progesterone designed to discriminate for ovulation (Shepard and

Senturia, 1977) indicated that ovulation was significantly more frequent in the Org 31710 group ($P = 0.005$; χ^2 test). In six out of seven volunteers in which a serum progesterone >10 nmol/l was found, sonographic signs for ovulation were present. In the remaining subject one ovary could not be visualized 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; oestradiol peak 664 ± 237 pmol/l; maximum progesterone concentrations were 26.0 ± 11.0 nmol/l (mean \pm SD). Ovulation was mostly observed around mid-cycle, i.e. in between monthly Org 31710 or placebo administration: day 8, 9, 10, 11, 12 (two subjects, one without ultrasound confirmation) and day 13. In seven women (three placebo, four 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 oestradiol concentrations.

In Figure 1 parameters of pituitary–ovarian activity are displayed during the monitored medication cycle of both groups. 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 days 1 and 2, 3 and 4, etc.). LH concentrations were significantly higher in the Org 31710 group on day 9/10 ($P < 0.001$) and 11/12 ($P = 0.002$). Furthermore, oestradiol concentrations were significantly lower in the Org 31710 group on days 3 and 4 ($P = 0.04$). Although mean progesterone values were higher ‘mid-cycle’ in the Org 31710 group due to the seven 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). Figure 2 shows that significant differences were found on day 9 for follicle diameter ($P = 0.05$) and oestradiol concentration ($P = 0.01$), on day 21 for LH concentration ($P = 0.001$) and on days 13 to 21 for progesterone ($P < 0.01$). 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 3). Oestradiol concentrations remained significantly different on day 3 and LH concentrations on days 9 and 11 as was observed in the complete Org 31710 versus placebo groups. This indicates that the observed differences in oestradiol and LH between Org 31710 and placebo were not only related to the occurrence of ovulation.

Endometrial thickness

The endometrium was significantly thicker in the Org 31710 group on cycle days 7 to 13 and cycle day 19 compared to the placebo group (Figure 4a). There was a weak but significant correlation between oestradiol concentrations 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 4b). Comparing all non-ovulatory volunteers, endometrial thickness again showed statistically significant differences on cycle days 7 to 13 and cycle day 19 ($P < 0.01$; Figure 4c).

Discussion

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

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 postulated to be affected through progesterone receptors in the theca layer (Hild-Petito *et al.*, 1988). Serum FSH concentrations seem to be less affected (Tafurt *et al.*, 1980), but generally peak concentrations 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 μ g 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 μ g DSG POP (Rice *et al.*, 1996). However, comparable concentrations of FSH, LH, follicle diameter, and oestradiol were observed in the Org 31710 and placebo groups. The data in this study corroborate the observations that although gonadotrophin peak concentrations are suppressed, dominant follicles commonly emerge and moderate oestradiol concentrations are present during POP therapy.

There were no statistically significant differences in overall (mean concentrations throughout a single cycle) or maximum concentrations of LH, FSH, oestradiol, and follicle diameter comparing Org 31710 users who did or did not ovulate. Furthermore, differences in sequential oestradiol concentrations and follicle diameter only reached statistical significance on day 9 and for LH on day 21. Although there was a tendency

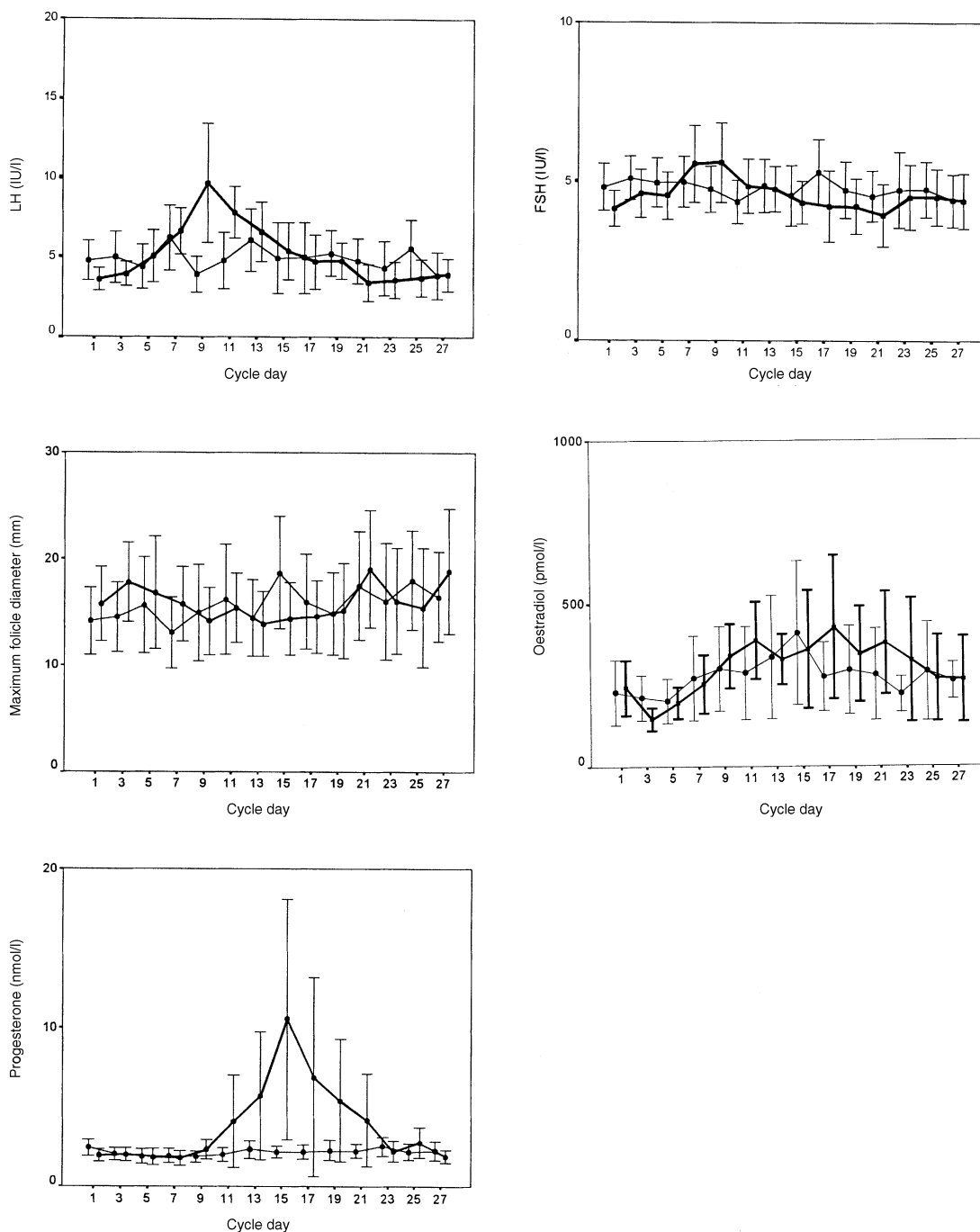


Figure 1. Pituitary-ovarian activity (mean \pm 95% confidence interval) in 75 μ g desogestrel + 150 mg Org 31710 group (—, $n = 25$) versus 75 μ g desogestrel + placebo group (---, $n = 23$). Each parameter was pooled for 2 consecutive cycle days (i.e. the first data set from cycle days 1 and 2, 3 and 4, etc.). LH = luteinizing hormone; FSH = follicle stimulating hormone.

for ovulation to occur at midcycle (i.e. in between Org 31710 doses), 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 oestradiol concentrations were statistically significantly 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 postulated 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 oestradiol-mediated feedback mechanisms, resulting in increased follicular sensitiv-

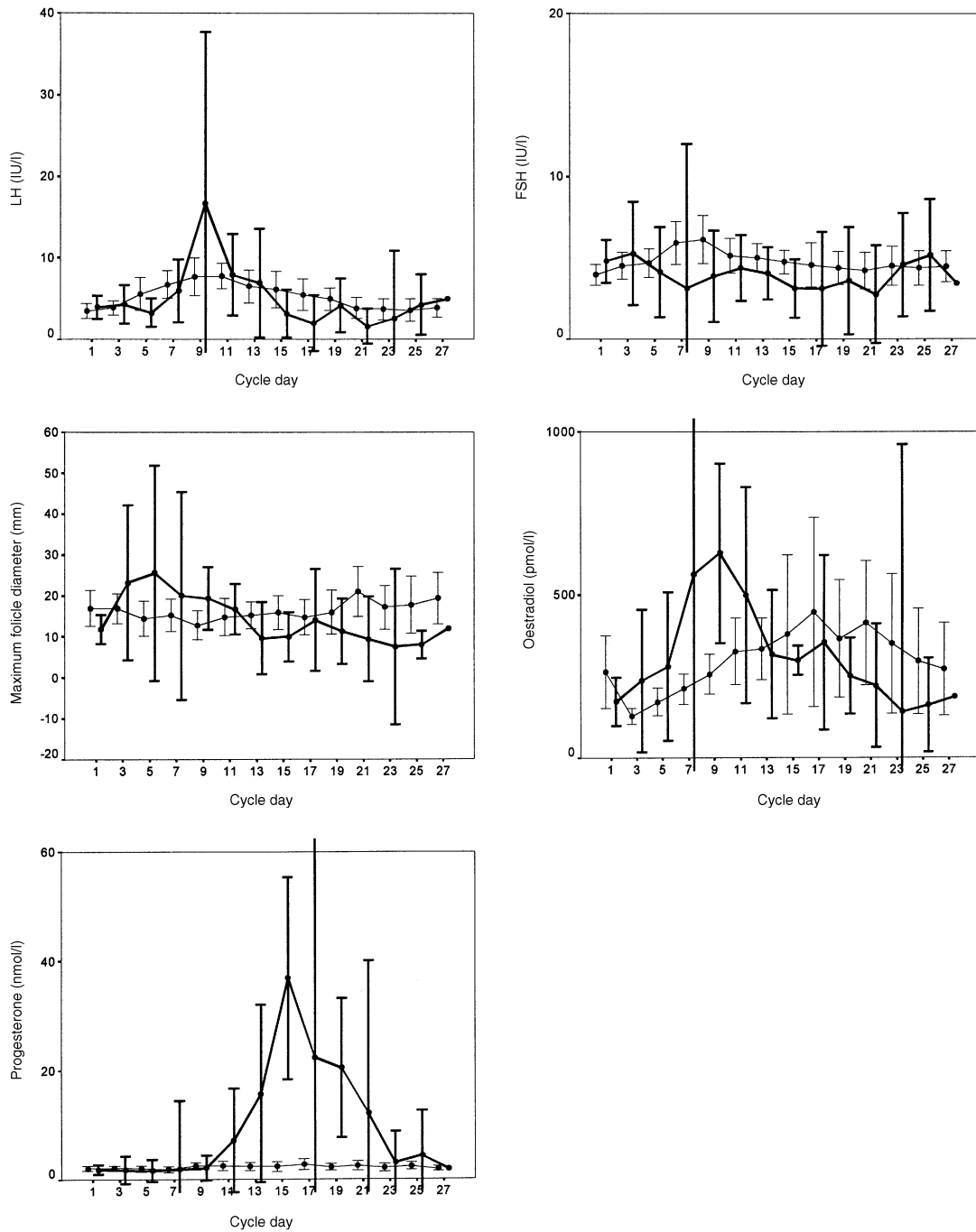


Figure 2. Pituitary-ovarian activity (mean \pm 95% confidence interval) in ovulatory desogestrel + Org 31710 group (—, $n = 7$) versus non-ovulatory desogestrel + Org 31710 group (---, $n = 18$). Each parameter was pooled for 2 consecutive cycle days (i.e. the first data set from cycle days 1 and 2, 3 and 4, etc.).

ity 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 concentration of the pituitary but rather at the hypothalamus (Heikinheimo *et al.*, 1996). Cyclic administration of mifepristone alone in different regimens showed partial inhibition of oestradiol secretion and suppressed progesterone concentrations (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 concentrations of gonadotrophins remained unchanged (Baird *et al.*, 1995). Ovulation was delayed by approximately 7 days. These findings support the hypothesis

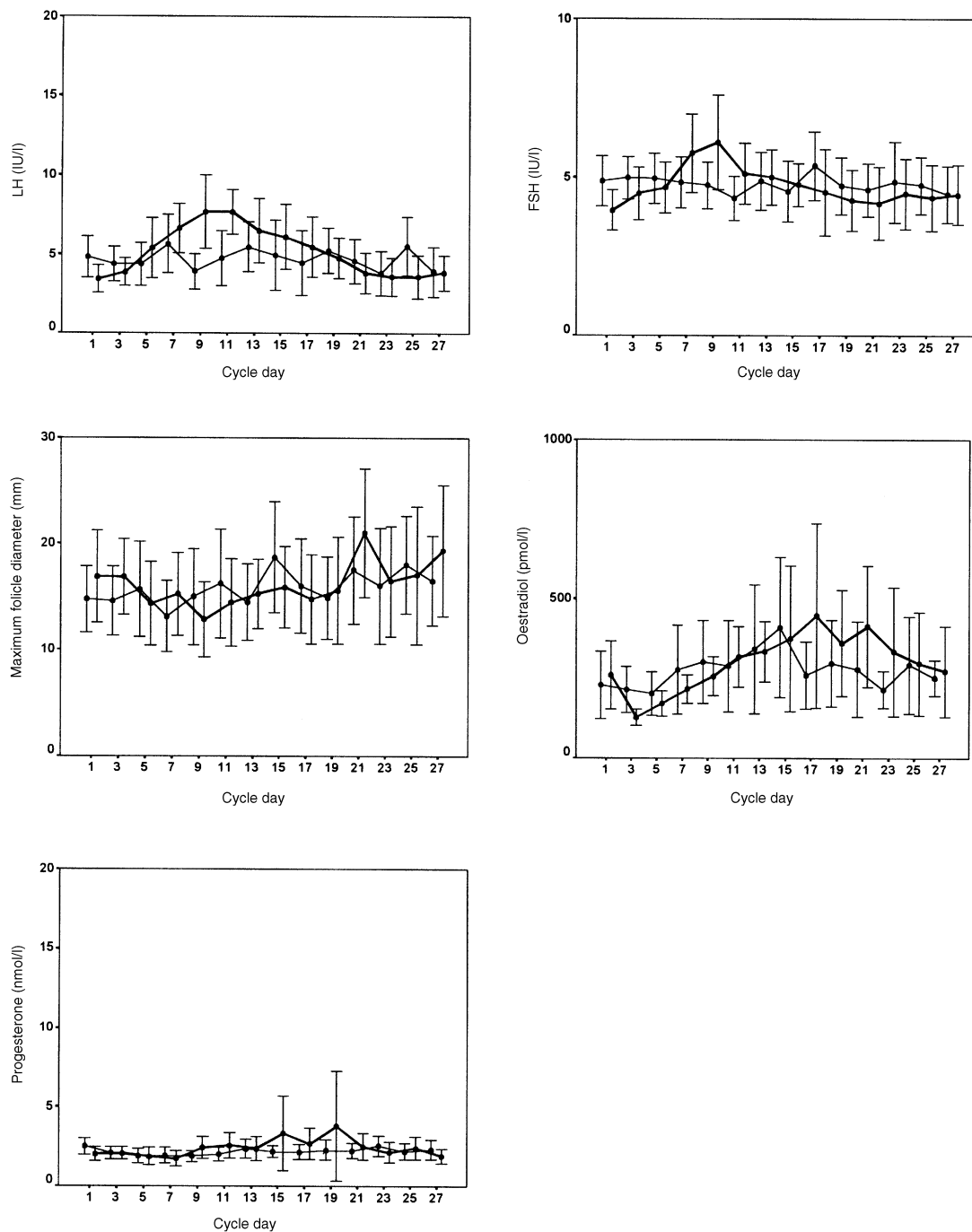


Figure 3. Pituitary–ovarian activity (mean \pm 95% confidence interval) in non-ovulatory desogestrel + Org 31710 group (—, $n = 18$) versus desogestrel + placebo group (---, $n = 23$). Each parameter was pooled for 2 consecutive cycle days (i.e. the first data set from cycle day 1 and 2, 3 and 4, etc.).

that a direct effect of an anti-progestagen on hypothalamic–pituitary regulation could be responsible for the observation that ovulation clustered around midcycle in the current 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 concentrations (Kim Bjorklund *et al.*, 1991;

Landgren *et al.*, 1994; Darney *et al.*, 1996), whereas others indicate that there might well be a relationship 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-oestrogenic activity (Van Uem *et al.*, 1989). This action was found to be dose-dependent in the presence of physiological concentrations of

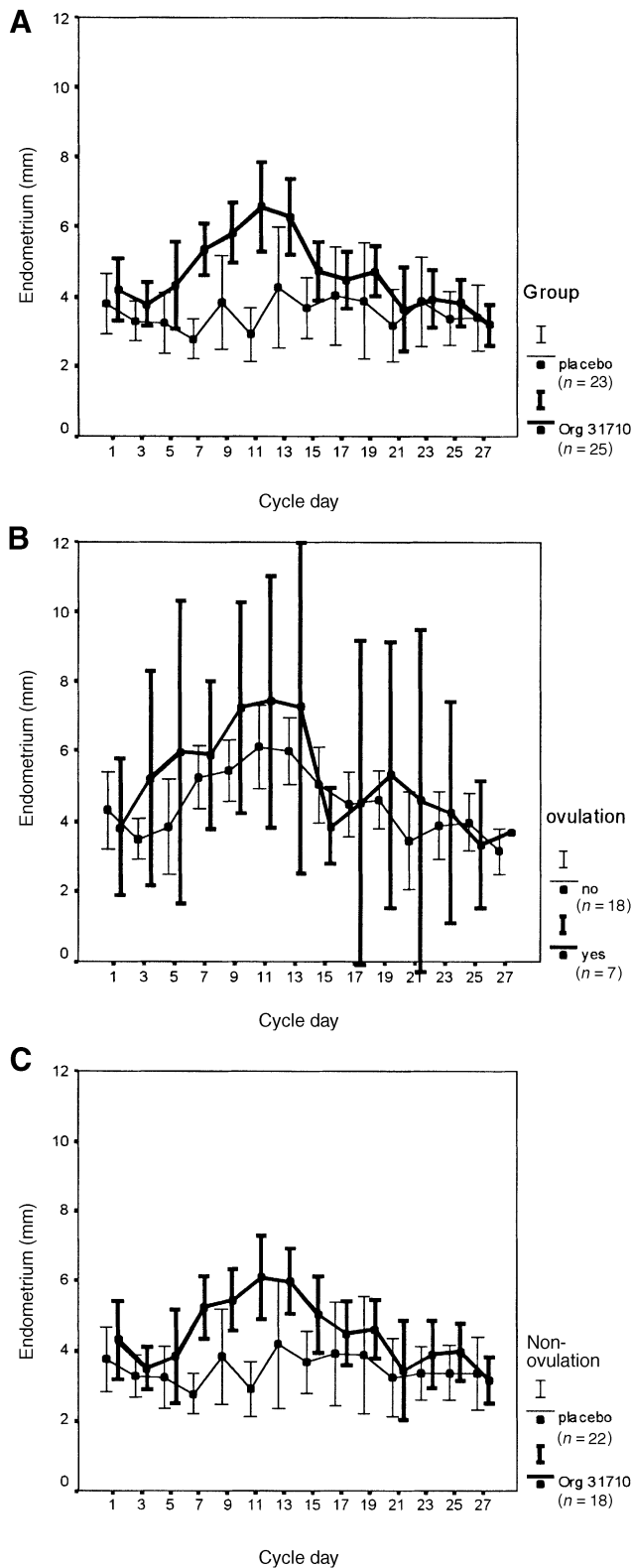


Figure 4. Endometrial thickness (mm, mean \pm 95% confidence interval) measured by transvaginal ultrasound in (A) desogestrel + Org 31710 versus desogestrel + placebo users; (B) ovulatory versus non-ovulatory desogestrel + Org 31710 users; (C) non-ovulatory desogestrel + Org 31710 versus desogestrel + placebo users. Each parameter was pooled for 2 consecutive cycle days (i.e. the first data set from cycle days 1 and 2, 3 and 4, etc.).

oestradiol. Mifepristone was antagonistic in the presence of progesterone, but demonstrated endometrial progestational effects at low doses and an antiproliferative (anti-oestrogenic) effect at higher doses in the absence of progesterone (Wolf *et al.*, 1989). 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 (results will be reported separately).

Continuous administration of mifepristone 1 mg/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 oestradiol and progesterone receptor concentrations (Neulen *et al.*, 1996) and the oestradiol receptor of the endometrial stroma alone have been reported (Murphy *et al.*, 1995). In this study, a statistically significant increase of endometrial thickness was found following the use of Org 31710, irrespective of the occurrence of ovulation. This difference gained statistical significance on days 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 oestradiol concentrations. 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 the influence of a single monthly dose of the anti-progestagen Org 31710 was assessed during a 75 μ g DSG continuous regimen of POP. The data suggest that Org 31710 may temporarily decrease oestradiol production, presumably through a direct effect on ovarian function. Furthermore, it may disrupt oestradiol-related feedback mechanisms through an effect at the hypothalamic-pituitary level and delay the LH surge. This may increase the likelihood of ovulation occurring during the POP cycles studied. Finally, there appears to be a direct effect on endometrial proliferation. Comparisons of the effects of Org 31710 with known mifepristone effects should be made with caution 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.

Acknowledgements

We would like to thank the staff of the Clinical Research Unit, Dijkzigt Hospital for their logistic support. We acknowledge the

effort of Dr T.M.T. Mulders for her work on the protocol. This study was financially supported by N.V. Organon and Stichting Voortplantingsgeneeskunde Rotterdam.

References

- Baird, D.T., Thong, K.J., Hall, C. and Cameron, S.T. (1995) Failure of oestrogen induced luteinizing hormone surge in women treated with mifepristone (RU 486) every day for 30 days. *Hum. Reprod.*, **10**, 2270–2276.
- Batista, M.C., Bristow, T.L., Mathews, J. *et al.* (1991) Daily administration of the progesterone antagonist RU 486 prevents implantation in the cycling guinea pig. *Am. J. Obstet. Gynecol.*, **165**, 82–86.
- Batista, M.C., Cartledge, T.P., Zellmer, A.W. *et al.* (1992) Delayed endometrial maturation induced by daily administration of the antiprogesterin RU 486: A potential new contraceptive strategy. *Am. J. Obstet. Gynecol.*, **167**, 60–65.
- Belsey, E.M. (1988) The association between vaginal bleeding patterns and reasons for discontinuation of contraceptive use. *Contraception*, **38**, 207–225.
- Broome, M. and Fotherby, K. (1990) Clinical experience with the progestogen-only pill. *Contraception*, **42**, 489–495.
- Darney, P.D., Taylor, R.N., Klaisle, C. *et al.* (1996) Serum concentrations of estradiol, progesterone, and levonorgestrel are not determinants of endometrial histology or abnormal bleeding in long-term Norplant registered implant users. *Contraception*, **53**, 97–100.
- De Jong, F.H., Baird, D.T. and van der Molen, H. (1974) Ovarian secretion rates of oestrogens, androgens and progesterone in normal women and in women with persistent ovarian follicles. *Acta Endocrinology*, **77**, 3–87.
- Heikinheimo, O., Gordon, K., Williams, R.F. and Hodgen, G.D. (1996) Inhibition of ovulation by progestin analogs (agonists versus antagonists): preliminary evidence for different sites and mechanisms of actions. *Contraception*, **53**, 55–64.
- Hemrika, D.J. (1993) Hypothalamic-pituitary-ovarian dynamics in oral contraceptive users. Thesis, Amsterdam.
- Hild-Petito, S., Stouffer, R.L. and Brenner, R.M. (1988) Immunocytochemical localization of estradiol and progesterone receptors in the monkey ovary throughout the menstrual cycle. *Endocrinology*, **123**, 2896–2905.
- Hodgen, G.D., Van Uem, J.F.H.M., Chillik, C.F. *et al.* (1994) Non-competitive anti-oestrogenic activity of progesterone antagonists in primate models. *Hum. Reprod.*, **9**, 77–81.
- Ishwad, P.C., Katkam, R.R., Hinduja, I.N. *et al.* (1993) Treatment with a progesterone antagonist ZK 98.299 delays endometrial development without blocking ovulation in bonnet monkeys. *Contraception*, **48**, 57–70.
- Kekkonen, R., Croxatto, H.B., Lahteenmaki, P. *et al.* (1995) Effects of intermittent antiprogesterin RU486 combined with cyclic medroxyprogesterone acetate on folliculogenesis and ovulation. *Hum. Reprod.*, **10**, 287–292.
- Kim Bjorklund, T., Landgren, B. and Hamberger, L. (1992) Is the contraceptive effect of 300 mcg of norethisterone mainly peripheral or central? *Contraception*, **45**, 57–66.
- Kim Bjorklund, T., Landgren, B.M. and Johannisson, E. (1991) Morphometric studies of the endometrium, the Fallopian tube and the corpus luteum during contraception with the 300 mcg norethisterone (NET) minipill. *Contraception*, **43**, 459–474.
- Kloosterboer, H.J., Deckers, G.H. and Schoonen, W.J. (1994) Pharmacology of two new very selective antiprogesteragens: Org 31710 and Org 31806. *Hum. Reprod.*, **9**, 47–52.
- Landgren, B.M. and Diczfalussy, E. (1980) Hormonal effects of the 300 mcg norethisterone (NET) minipill. 1. Daily steroid levels in 43 subjects during a pretreatment cycle and during the second month of NET administration. *Contraception*, **21**, 87–113.
- Landgren, B.M., Balogh, A., Shin, M.W. *et al.* (1979) Hormonal effects of the 300 mcg norethisterone (NET) minipill. 2. Daily gonadotrophin levels in 43 subjects during a pretreatment cycle and during the second month of NET administration. *Contraception*, **20**, 585–605.
- Landgren, B.M., Aedo, A.R., Johannisson, E. and Cekan, S.Z. (1994) Pharmacokinetic and pharmacodynamic effects of vaginal rings releasing levonorgestrel at a rate of 27 mcg g/24 h: A pilot study. *Contraception*, **49**, 139–150.
- McCann, M.F. and Potter, L.S. (1994) Progestin-only oral contraception: A comprehensive review. *Contraception*, **50**, S1–S198.
- Mizutani, T., Bhakta, A., Kloosterboer, H.J. and Moudgil, V.K. (1992) Novel antiprogesterins Org 31806 and 31710: Interaction with mammalian progesterone receptor and DNA binding of antisteroid receptor complexes. *J. Steroid Biochem. Mol. Biol.*, **42**, 695–704.
- Murphy, A.A., Kettel, L.M., Morales, A.J. *et al.* (1995) Endometrial effects of long-term low-dose administration of RU486. *Fertil. Steril.*, **63**, 761–766.
- Neulen, J., Williams, R.F., Breckwoldt, M. *et al.* (1996) Non-competitive anti-oestrogenic actions of progesterone antagonists in primate endometrium: enhancement of oestrogen and progesterone receptors with blockade of post-receptor proliferative mechanisms. *Hum. Reprod.*, **11**, 1533–1537.
- Pache, T.D., Wladimiroff, J.W., De Jong, F.H. *et al.* (1990) Growth patterns of nondominant ovarian follicles during the normal menstrual cycle. *Fertil. Steril.*, **54**, 638–642.
- Rice, C., Killock, S., Hickling, D. and Coelingh, B.H. (1996) Ovarian activity and vaginal bleeding patterns with a desogestrel-only preparation at three different doses. *Hum. Reprod.*, **11**, 737–740.
- Shaaban, M.M., Segal, S., Salem, H.T. *et al.* (1993) Sonographic assessment of ovarian and endometrial changes during long-term Norplant use and their correlation with hormonal levels. *Fertil. Steril.*, **59**, 998–1002.
- Shepard, M.K. and Senturia, Y.D. (1977) Comparison of serum progesterone and endometrial biopsy for confirmation of ovulation and evaluation of luteal function. *Fertil. Steril.*, **28**, 5–8.
- Shoupe, D., Horenstein, J., Mishell, D.R. *et al.* (1991) Characteristics of ovarian follicular development in Norplant users. *Fertil. Steril.*, **55**, 766–770.
- Spitz, I.M., Croxatto, H.B., Salvatierra, A.M. and Heikinheimo, O. (1993) Response to intermittent RU486 in women. *Fertil. Steril.*, **59**, 971–975.
- Tafurt, C.A., Sobrevilla, L.A. and De Estrada, R. (1980) Effects of progestin-estrogen combination and progestational contraceptives on pituitary gonadotropins, gonadal steroids, and sex hormone-binding globulin. *Fertil. Steril.*, **33**, 261–266.
- Tayob, Y., Guillebaud, J., Adams, J. and Jacobs, H.S. (1986) Studies on ovarian function in users of the progestagen only contraceptive pill. *J. Obstet. Gynecol.*, **6**, S91–S95.
- van Santbrink, E.J., Hop, W.C., van Dessel, H.J. *et al.* (1995) Decremental follicle-stimulating hormone and dominant follicle development during the normal menstrual cycle. *Fertil. Steril.*, **64**, 37–43.
- Van Uem, J.F.H.M., Hsiu, J.G., Chillik, C.F. *et al.* (1989) Contraceptive potential of RU 486 by ovulation inhibition: I. Pituitary versus ovarian action with blockade of estrogen-induced endometrial proliferation. *Contraception*, **40**, 171–184.
- Wolf, J.P., Hsiu, J.G., Anderson, T.L. *et al.* (1989) Noncompetitive antiestrogenic effect of RU 486 in blocking the estrogen-stimulated luteinizing hormone surge and the proliferative action of estradiol on endometrium in castrate monkeys. *Fertil. Steril.*, **52**, 1055–1060.
- Zalanyi, S., Landgren, B. and Johannisson, E. (1984) Pharmacokinetics, pharmacodynamic and endometrial effects of a single dose of 200 mg norethisterone enanthate. *Contraception*, **30**, 225–237.

Received on August 6, 1999; accepted on November 25, 1999