ORIGINAL RESEARCH

Endometrial safety of an oral contraceptive containing estradiol valerate and dienogest

Johannes Bitzer[1]
Susanne Parke[2]
Thomas Roemer[3]
Marco Serrani[2]

[1]University Hospital of Basel, Women's Clinic, Basel, Switzerland; [2]Global Clinical Development, Bayer HealthCare Pharmaceuticals, Berlin, Germany; [3]Head of the Department of Gynecology and Obstetrics, Evangelist Hospital Cologne-Weyertal, Cologne, Germany

Background: The purpose of this study was to investigate the endometrial safety of an oral contraceptive containing estradiol valerate/dienogest (E_2V/DNG) administered as an estrogen step-down and progestogen step-up regimen in women of reproductive age (18–50 years), using histological assessment of endometrial biopsy samples.

Methods: Endometrial biopsies were taken in a subset of healthy women who took part in a multicenter, open-label, noncomparative study assessing the contraceptive efficacy and safety of an E_2V/DNG oral contraceptive. In each 28-day cycle, women received E_2V 3 mg on days 1–2, E_2V 2 mg/DNG 2 mg on days 3–7, E_2V 2 mg/DNG 3 mg on days 8–24, E_2V 1 mg on days 25–26, and placebo on days 27–28. Women underwent endometrial biopsy between days 12 and 19 of the cycle, both at screening and at cycle 20 (or at the final examination).

Results: Of the 283 women who underwent an endometrial biopsy at screening, 218 underwent a follow-up biopsy at cycle 20. At screening, abnormal biopsy results, both classified as "simple hyperplasia without atypia", were seen in two women, who were withdrawn from the trial. At cycle 20, there were no abnormal findings of endometrial hyperplasia or malignancy, and 80.9% of women had atrophic, inactive, or secretory endometrium.

Conclusion: After 20 cycles of treatment, an oral contraceptive containing E_2V and DNG is safe and effective to transform the endometrium into a secretory/inactive or atrophic status, and exhibits no deleterious effects on endometrial histology in women aged 18–50 years.

Keywords: estradiol valerate, dienogest, endometrial safety, oral contraceptive

Introduction

The use of combined oral contraceptives is associated with distinct changes in the histology of the endometrium. Such changes include the inhibition of glandular growth and differentiation, resulting in an inactive or atrophic endometrium in many users.[1–4] In addition to inhibition of ovulation, these changes contribute to the contraceptive efficacy of combined oral contraceptives, creating a thin, flat endometrium. While combined oral contraceptives contain both an estrogen and a progestogen component, the overall effect of combined oral contraceptives on the endometrium is considered to be attributable to the progestogen component,[5,6] the effects of which are mediated via the progesterone receptor.[7]

Dienogest (DNG) is one of several new progestogens that have been introduced in recent decades.[8] It is a specific progesterone receptor agonist[7] and combines the properties of both 19-nortestosterone derivatives and progesterone derivatives.[9] Consequently, DNG has a unique pharmacological profile and a pronounced effect on the endometrium.[7,9,10]

Correspondence: Susanne Parke
Bayer HealthCare Pharmaceuticals,
Müllerstrasse 178, 13342 Berlin,
Germany
Tel +49 30 4681 2634
Fax +49 30 4681 5348
Email susanne.parke@bayer.com

127

DNG 2 mg (Visanne®; Bayer HealthCare Pharmaceuticals, Germany) is indicated for the treatment of endometriosis. At this same dose, DNG also represents the progestogen component of a combined oral contraceptive containing ethinylestradiol 30 μg and of a continuous hormone replacement therapy containing estradiol valerate (E_2V) 2 mg. Both preparations are highly effective in their respective indications, and are well tolerated and associated with a favorable bleeding profile.[11–14] Attempts to develop estradiol-containing combined oral contraceptives have historically been characterized by suboptimal cycle stability and bleeding irregularities, particularly when administered as monophasic or biphasic regimens,[15–19] although excellent contraceptive efficacy in clinical studies has been achieved.[15–20] More recently, a novel oral contraceptive comprising DNG and E_2V in a dynamic estrogen step-down and progestogen step-up dosing regimen has become available in many countries worldwide. This regimen comprises an extended hormone-containing phase (26 days), including four days of unopposed estrogen delivery, and a shortened hormone-free interval (two days). The $E_2V/$DNG oral contraceptive has been shown in clinical trials to be an effective contraceptive that is well tolerated and associated with a good bleeding profile.[21,22]

This study was designed to investigate the contraceptive efficacy, safety, and tolerability of the E_2V/DNG regimen in 1377 women of reproductive age, using the preparation as an oral contraceptive; the primary findings of this trial have previously been reported.[22] The E_2V/DNG regimen was associated with an overall unadjusted Pearl Index of 0.73 and a well-controlled and regular bleeding pattern. E_2V/DNG was well tolerated and mean body weight generally remained stable throughout the study visits. A large majority of the women reported unchanged or improved physical and emotional well-being, compared with their pretreatment status, as well as a high degree of overall satisfaction with the study drug.

The importance of investigating endometrial changes in women using new combined oral contraceptives as indicators for the development of endometrial cancer is reflected in the European Medicines Agency Guideline on Clinical Investigations of Steroid Contraceptives in Women.[23] Therefore, histological assessment of endometrial biopsy samples was undertaken in a subgroup of women aged 18–50 years to assess the endometrial safety of E_2V/DNG.

Methods
Design and participants
The full methodological details of this trial have previously been reported.[22] Briefly, this was a multicenter, open-label, noncomparative study of E_2V/DNG, administered using an estrogen step-down and progestogen step-up dosing regimen. To assess the effect of the E_2V/DNG regimen on the endometrium, endometrial histology was assessed in a subgroup of women from selected study centers who took part in this trial.

Healthy female volunteers (aged 18–50 years) requesting contraception, who had a body mass index of ≤30 kg/m² and a normal cervical smear test result, were included in this trial. Participants in the study were either new users of hormonal contraception or had switched from another oral contraceptive. Exclusion criteria were generally consistent with the usual contraindications for combined oral contraceptive use. The use of long-acting hormonal contraception immediately prior to the study was not permitted. In addition, no endometrial biopsies were taken from women with an acute genital infection, as diagnosed at the discretion of the investigator. Women in the endometrial biopsy subgroup discontinued the study if the screening biopsy revealed relevant pathology.

Treatment
Women participating in this trial received treatment for a total of 20 cycles. Each 28-day cycle comprised E_2V 3 mg on days 1–2, E_2V 2 mg/DNG 2 mg on days 3–7, E_2V 2 mg/DNG 3 mg on days 8–24, E_2V 1 mg on days 25–26, and placebo on days 27–28. One tablet was taken daily with no tablet-free interval between cycles. In the first treatment cycle, the first tablet was taken on the first day of menstrual or withdrawal bleeding.

Assessments
Endometrial biopsies were taken between days 12 and 19 of the cycle, both at screening (including during active use of another oral contraceptive prior to switching to treatment with E_2V/DNG at visit 2) and at cycle 20 (or at a final examination if women prematurely discontinued the study between cycles 6 and 20). If insufficient tissue was obtained, the biopsy was repeated within four weeks.

Endometrial biopsies were taken with a pipelle and did not require dilation of the cervix or anesthesia. Endometrial material was obtained from the anterior or posterior wall of the fundus area. Material taken from the tubal corner or the endocervix was deemed inappropriate, and was not used. The Laboratorium für Klinische Forschung (LKF), Kiel, Germany was responsible for providing materials and organizing logistics. For the evaluation of endometrial biopsies, the specimens were sent to LKF. One pathologist

(reader) assessed the samples. Thereafter, the samples were stored at LKF to enable a rereading procedure at a later time point, if necessary. To evaluate the endometrial safety of E₂V/DNG, each endometrial biopsy sample was classified according to one of three categories, ie, insufficient material, normal/nonsuspicious, or abnormal. Histological categories for the evaluation of the endometrium are described in Table 1.

Statistical analysis

For this prospectively defined subgroup analysis, endometrial biopsies were planned to be taken in a subgroup of 250 women at screening and at the final examination. Taking into consideration a dropout rate of 40% over 20 cycles, which is typically observed in long-term studies of oral contraceptives, approximately 150 biopsies were aimed to be available at cycle 20. No formal sample-size calculation for the number of biopsy samples was performed. Biopsy results are presented as a percentage of the total number of assessments.

Results
Subject disposition

A total of 1377 women were included in the study. Endometrial biopsies were taken in 283 of these women at screening. An endometrial biopsy was undertaken in 218 of these women at a final examination. In this subgroup, 22.4%

of women were new oral contraceptive users and 67.3% were switchers from another oral contraceptive.

Endometrial histology

A summary of endometrial biopsy samples, classified by histological description, at screening and at the final examination is presented in Table 2. Abnormal biopsy findings at screening were seen in two women (both classified as "simple hyperplasia without atypia"). These women were therefore prematurely withdrawn from the study.

Of the normal endometrial biopsies observed at the screening visit, the majority were of the "proliferative" type. Just over one-third of biopsies (35.4%) were "atrophic" or "inactive". At the final examination, there were no abnormal findings of endometrial hyperplasia or malignancy. Normal endometrial biopsy results were seen in 204 women (93.2%). Nonassessable results were recorded in 14 women (6.4%). Overall, 24.7% of the normal biopsies at the final examination time point were "atrophic", 39.3% were "inactive", 11.4% were of the "proliferative" type (weakly [8.2%], disordered [1.8%], or actively [1.4%]), 16.9% were of the "secretory" type (progestational [10.5%] or cyclic [6.4%]), and 0.5% were of the "menstrual" type. In total, 80.9% of women had an atrophic, inactive or secretory endometrium.

Table 1 Histological categories for evaluation of endometrial biopsies

Histological categories		
Insufficient material	**Normal biopsy results**	**Abnormal biopsy results**
No tissue	Atrophic[a]	Simple hyperplasia without atypia
Tissue insufficient for diagnosis	Inactive	Simple hyperplasia with atypia
	Proliferative	Complex hyperplasia without atypia
	Weakly proliferative	
	Actively proliferative[b]	
	Disordered proliferative[c]	
	Secretory	Complex hyperplasia with atypia
	Cyclic type	
	Progestational type	
	Menstrual type	Carcinoma

Notes: [a]Includes cases with sufficient atrophic epithelial strips without intact glands or stroma; [b]Actively proliferative endometrium has numerous mitoses, but lacks secretory or metaplastic changes; [c]Disordered proliferative endometrium describes the cystic dilation of randomly scattered glands dispersed within the array of normally present proliferative cells, gland density is not changed and occasional glands may have some branching. This differs from endometrial hyperplasia, which is defined as proliferation of glands of irregular size and shape with an accompanying increase in the glands:stroma ratio.[24]

Table 2 Endometrial biopsy diagnosis, classified by histological description, at screening and at final examination (full biopsy analysis set)

	Screening (visit 1) (n = 283)		Final examination (visit 8) (n = 219)	
	n	(%)	n	(%)
Normal endometrium	253	89.4	204	93.2
Atrophic	50	17.7	54	24.7
Inactive	50	17.7	86	39.3
Proliferative				
Weakly proliferative	29	10.2	18	8.2
Actively proliferative	15	5.3	3	1.4
Disordered proliferative	81	28.6	4	1.8
Secretory				
Cyclic type	16	5.7	14	6.4
Progestational type	6	2.1	23	10.5
Menstrual type	1	0.4	1	0.5
Abnormal endometrium	2	0.7	0	0
Not assessable	28	9.9	14	6.4
Not taken	0	0	1	0.5

There were five women with a diagnosis of endometrial polyps at screening. The polyps were classified as "atrophic" in three women and as "functional" in two women. In all five women, a biopsy was taken at the final examination. There were no women with signs of endometrial polyps at the final examination. Signs of limited focal endometrial metaplasia on a background of inactive endometrium were observed in four women overall; two women at the time of screening and two at the final examination. In all cases, there were no abnormal findings of endometrial hyperplasia.

Discussion

The results of this prospectively defined subgroup analysis demonstrate the endometrial safety of E_2V/DNG administered in an estrogen step-down and progestogen step-up regimen, including four days of unopposed estrogen. After 20 cycles of treatment, endometrial biopsies revealed an inactive or atrophic endometrium in 64% of women. Of note, no findings of hyperplasia or malignancy were observed during 20 cycles of treatment.

Estrogen exerts a stimulatory effect on the endometrium, and long-term unopposed exposure to even low doses is associated with a substantial risk of abnormal endometrial growth, as has been extensively reviewed previously.[24] Indeed, the administration of unopposed estrogen to postmenopausal women has been associated with an increased risk of endometrial hyperplasia[25] and endometrial cancer.[26] Although the E_2V/DNG oral contraceptive used in the current study includes four days (days 1–2 and days 25–26) of unopposed E_2V, there was no evidence of an increase in the incidence of endometrial hyperplasia. These results support data from previous studies of combined oral contraceptives, which did not observe any changes that could be described as hyperplasic or malignant.[27–30]

The stimulatory effect of estrogen on the endometrium is opposed by progestogens, which inhibit estrogen-induced endometrial proliferation. In the current subgroup analysis, good suppression of endometrial activity was observed. Furthermore, endometrial suppression was evident at a time point of the treatment cycle between day 12 and day 19, when the proliferative stimulation by the estrogenic compound is expected to approach its maximum level. DNG is a selective progesterone receptor agonist that has potent endometrial activity when administered orally.[7] DNG exerts a potent progestogenic effect at the level of the endometrium, counteracting estrogenic activity. In postmenopausal women receiving estrogen, this results in protection of the endometrium.[31] Furthermore, it is important to know the effect of a new contraceptive formulation on the endometrium because endometrial function also plays an important role in the uterine bleeding process.

The results of the current subgroup analysis are comparable with those of a limited number of studies that have assessed the endometrial effects of other cyclic or extended low-dose combined oral contraceptives.[2,4,27–30] In a study by Anderson et al, women received a 91-day extended regimen of ethinylestradiol 30 µg/levonorgestrel 150 µg that provided low-dose estrogen (10 µg) instead of placebo during the typical seven-day hormone-free interval.[27] During active treatment, 70% of women had an inactive or atrophic endometrium. Similarly, after 13 cycles of treatment with ethinylestradiol 30 µg/drospirenone 3 mg in a 21-day regimen with a seven-day tablet-free period, 63% of women had an atrophic endometrium.[2] Also, histological and ultrasonographical evaluations confirmed that the proliferative activity of the endometrium was suppressed by this oral contraceptive treatment. Furthermore, no adverse endometrial effects were observed after 13 cycles of a continuous combined oral contraceptive regimen (ethinylestradiol 20 µg/levonorgestrel 90 µg); 52% of women receiving this regimen had endometrial histology results categorized as "other", which primarily included an inactive or benign endometrium.[29] In another study, no adverse endometrial effects were observed among women who received a 21-day ethinylestradiol 20 µg/desogestrel 150 µg regimen with a reduced hormone-free interval and five days of unopposed estrogen treatment (placebo was administered on days 22–23, followed by ethinylestradiol 10 µg on days 24–28).[28] In a recent study by Rabe et al, 59 women underwent endometrial biopsy to examine the effect of ethinylestradiol 0.02 mg/chlormadinone acetate 2 mg administered in a 24/4-day regimen on endometrial histology. After six treatment cycles, ethinylestradiol 0.02 mg/chlormadinone acetate 2 mg demonstrated effective and reversible endometrial effects without exerting any pathological effects. At cycle 6, 16% of subjects had a secretory and 60% an inactive endometrium.[30]

The use of estradiol as the only estrogen component in combined oral contraceptives has so far not been successful because it has been characterized by suboptimal cycle stability and bleeding irregularities, particularly when administered as monophasic or biphasic regimens.[15–19] This can be explained by local enzymes (eg, 17ß-hydroxysteroid dehydrogenase) which, in the presence of a progestogen, are stimulated within the endometrial cells, causing rapid oxidation of estradiol to estrone.[32] Therefore, the local level of estradiol is also

reduced, which may cause the incidence of intermenstrual bleeding to increase. Furthermore, sequential preparations are known to improve cycle stability in women experiencing bleeding irregularities. Early estrogenic dominance is hypothesized to ensure initial endometrial proliferation and sensitivity for mid-cyclic progestogen action and endometrial stroma stability, particularly towards the end of the cycle.[21] To ensure estrogen dominance in the first cycle phase, ie, the stage at which the proliferation of the endometrium is promoted under estrogen influence, E_2V was administered on days 1–26 of the cycle at doses stepped down from 3 mg to 1 mg. Essentially, the shortened hormone-free interval to only two days, the progestogen dominance in the mid-to-late part of the cycle, and the estrogen-only phase before and at the end of the progestogen phase were expected to increase the cycle stability.

It has been shown previously that E_2V/DNG in the present formulation is associated with a bleeding pattern and cycle control profile that is comparable with that of a low-dose ethinylestradiol-containing oral contraceptive.[21] In the present study, the overall frequency of women experiencing withdrawal bleeding was relatively stable throughout the treatment cycles, ranging between 76.8% and 81.6% during cycles 1–19. Furthermore, only 0.1% of 1377 women discontinued the study medication prematurely due to the absence of withdrawal bleeding.

The number of women with intracyclic bleeding decreased during the course of the study from 24.0% during cycle 2 and reaching the lowest level of 10.2% during cycle 18. In general, there were fewer women with intracyclic bleeding episodes during the second half of the study compared with the first half of the treatment cycles. Only 2.5% of women prematurely discontinued study medication due to menstrual bleeding irregularities.

The results of this analysis are reassuring on several counts. Firstly, this is one of the largest and longest studies assessing endometrial histology with combined oral contraceptives in women of childbearing age, and the study was conducted over a period of up to 20 cycles. Also, this study population included women aged 18–50 years and, therefore, included women in their late reproductive years who might be at an increased risk of endometrial pathology.

At screening, almost one-half of the normal biopsy samples were "proliferative", while one-third of normal samples were "atrophic" or "inactive". This reflects the heterogeneous group of women that were included in the study, which consisted of new users of hormonal contraception (ie, spontaneously cycling women) and women switching from other oral contraceptive. At the final examination, only 11.4% of the normal biopsies were of the "proliferative" type.

Endometrial polyps were observed in five women at screening and in no women at the final examination. It is not possible to claim that the use of E_2V/DNG is associated with a decrease in endometrial polyps, but no additional cases of polyps were detected, suggesting that the preparation does not have a deleterious effect in this setting. It should be noted that it is not possible to ensure, by performing an endometrial biopsy, that each and every polyp had disappeared. However, small polyps are not a contraindication for the use of E_2V/DNG or low-dose oral contraceptives in general.

Consistent with results reported for other low-dose combined oral contraceptives, this prospectively defined subgroup analysis demonstrated that an oral contraceptive containing E_2V and DNG is safe and effective to transform the endometrium to a secretory/inactive or atrophic status and exhibits no deleterious effects on the endometrium of women aged 18–50 years during 20 cycles of treatment.

Acknowledgments

Disclosure

References

1. Oosterbaan HP. An open-label study of the effects of a 24-day regimen of gestodene 60 microg/ethinylestradiol 15 microg on endometrial histological findings in healthy women. *Eur J Contracept Reprod Health Care*. 1999;4 Suppl 2:3–8.
2. Ludicke F, Johannisson E, Helmerhorst FM, Campana A, Foidart J, Heithecker R. Effect of a combined oral contraceptive containing 3 mg of drospirenone and 30 microg of ethinyl estradiol on the human endometrium. *Fertil Steril*. 2001;76(1):102–107.
3. Anderson FD, Hait H, Hsiu J, Thompson-Graves AL, Wilborn WH, Williams RF. Endometrial microstructure after long-term use of a 91-day extended-cycle oral contraceptive regimen. *Contraception*. 2005;71(1):55–59.

4. Coenen CM, Hollanders JM, Rolland R, Spielmann D, Bulten J. The effects of a low-dose gestodene-containing oral contraceptive on endometrial histology in healthy women. *Eur J Contracept Reprod Health Care*. 1996;1(4):325–329.

5. Rowlands S. Newer progestogens. *J Fam Plann Reprod Health Care*. 2003;29(1):13–16.

6. Williams CL, Stancel GM. *Estrogens and Progestins*. 9th ed. New York, NY: McGraw-Hill; 1996.

7. Sasagawa S, Shimizu Y, Kami H, et al. Dienogest is a selective progesterone receptor agonist in transactivation analysis with potent oral endometrial activity due to its efficient pharmacokinetic profile. *Steroids*. 2008;73(2):222–231.

8. Sitruk-Ware R. New progestagens for contraceptive use. *Hum Reprod Update*. 2006;12(2):169–178.

9. Oettel M, Breitbarth H, Elger W, et al. The pharmacological profile of dienogest. *Eur J Contracept Reprod Health Care*. 1999;4 Suppl 1: 2–13.

10. Oettel M, Gräser T, Hoffmann H, Moore C, Zimmermann H, Zimmermann T. The preclinical and clinical profile of dienogest. A short overview. *Drugs Today*. 1999;35 Suppl C:3–12.

11. Golbs S, Domhardt R, Radowicky S, Kaluzny Z, Wisser KH, Zimmermann T. Clinical findings with the oral contraceptive combination ethinylestradiol/dienogest in Poland. *Methods Find Exp Clin Pharmacol*. 2002;24(9):585–592.

12. Graser T, Romer T, Wiedey KD, Janaud A. Climodien (estradiol valerate 2 mg plus dienogest 2 mg) is safe and effective in the treatment of postmenopausal complaints. *Climacteric*. 2001;4(4):332–342.

13. Von Schoultz B. Clinical efficacy and safety of combined estradiol valerate and dienogest: A new no-bleed treatment. *Climacteric*. 2003; 6 Suppl 2:24–32.

14. Zimmermann T, Dietrich H, Wisser KH, Hoffmann H. The efficacy and tolerability of Valette: A postmarketing surveillance study. *Eur J Contracept Reprod Health Care*. 1999;4(3):155–164.

15. World Health Organization Task Force on Oral Contraception. A randomized, double-blind study of two combined oral contraceptives containing the same progestogen, but different estrogens. *Contraception*. 1980;21(5):445–459.

16. Kivinen S, Saure A. Efficacy and tolerability of a combined oral contraceptive containing 17 beta-estradiol and desogestrel. *Eur J Contracept Reprod Health Care*. 1996;1:183.

17. Schubert W, Cullberg G. Ovulation inhibition with 17 beta-estradiol cyclo-octyl acetate and desogestrel. *Acta Obstet Gynecol Scand*. 1987; 66(6):543–547.

18. Serup J, Bostofte E, Larsen S, Westergaard J, Lebech PE. Natural oestrogens for oral contraception. *Lancet*. 1979;2(8140):471–472.

19. Wenzl R, Bennink HC, van Beek A, Spona J, Huber J. Ovulation inhibition with a combined oral contraceptive containing 1 mg micronized 17 beta-estradiol. *Fertil Steril*. 1993;60(4):616–619.

20. Hirvonen E, Vartiainen E, Kulmala Y. A multicenter trial with a new OC using a natural estradiol and cyproterone acetate for women over 35. *Adv Contracept*. 1990;6(4):248.

21. Ahrendt HJ, Makalova D, Parke S, Mellinger U, Mansour D. Bleeding pattern and cycle control with an estradiol-based oral contraceptive: A seven-cycle, randomized comparative trial of estradiol valerate/ dienogest and ethinyl estradiol/levonorgestrel. *Contraception*. 2009; 80(5):436–444.

22. Palacios S, Wildt L, Parke S, Machlitt A, Romer T, Bitzer J. Efficacy and safety of a novel oral contraceptive based on oestradiol (oestradiol valerate/dienogest): a Phase III trial. *Eur J Obstet Gynecol Reprod Biol*. 2010;149(1):57–62.

23. European Medicines Agency. Committee for Medicinal Products for Human Use. Guideline on clinical investigation of steroid contraceptives in women. EMEA/CPMP/EWP/519/98 Rev 1. London 2005:1–6. Available at: http://www.tga.gov.au/docs/pdf/euguide/ewp/051998enr1. pdf. Accessed March 09, 2011.

24. Montgomery BE, Daum GS, Dunton CJ. Endometrial hyperplasia: A review. *Obstet Gynecol Surv*. 2004;59(5):368–378.

25. Furness S, Roberts H, Marjoribanks J, Lethaby A, Hickey M, Farquhar C. Hormone therapy in postmenopausal women and risk of endometrial hyperplasia. *Cochrane Database Syst Rev*. 2009(2):CD000402.

26. Nelson HD, Humphrey LL, Nygren P, Teutsch SM, Allan JD. Postmenopausal hormone replacement therapy: Scientific review. *JAMA*. 2002;288(7):872–881.

27. Anderson FD, Feldman R, Reape KZ. Endometrial effects of a 91-day extended-regimen oral contraceptive with low-dose estrogen in place of placebo. *Contraception*. 2008;77(2):91–96.

28. Archer DF. Endometrial histology during use of a low-dose estrogen-desogestrel oral contraceptive with a reduced hormone-free interval. *Contraception*. 1999;60(3):151–154.

29. Johnson JV, Grubb GS, Constantine GD. Endometrial histology following 1 year of a continuous daily regimen of levonorgestrel 90 micro g/ethinyl estradiol 20 micro g. *Contraception*. 2007;75(1):23–26.

30. Rabe T, Hartschuh E, Wahlstrom T, Hoschen K, Konig S. Endometrial safety of a novel monophasic combined oral contraceptive containing 0.02 mg ethinylestradiol and 2 mg chlormadinone acetate administered in a 24/4-day regimen over six cycles. *Contraception*. 2010;82(4):358–365.

31. Graser T, Muller A, Mellinger U, Muck AO, Lippert TH, Oettel M. Continuous-combined treatment of the menopause with combinations of oestradiol valerate and dienogest – a dose-ranging study. *Maturitas*. 2000;35(3):253–261.

32. Kuhnz W, Blode H, Zimmermann H. Pharmacokinetics of exogenous natural and synthetic estrogens and antiestrogens. In: Oettel M, Schillinger E, editors. *Handbook of Experimental Pharmacology*. Berlin: Springer; 1999.