Dienogest offers pharmacological advantages for the effective treatment of endometriosis and for use in contraception and hormone replacement therapy. This pharmacodynamic study investigated the ovulation-inhibiting effects of dienogest monotherapy in healthy women. Dienogest was administered at 0.5, 1, 2, or 3 mg daily for up to 72 days to women aged 18 to 35 years (n = 102). Ovarian activity was assessed pretreatment and during 2 treatment periods (days 0-36 and days 37-72) by the Hoogland score, based on follicle size and serum estradiol and progesterone levels. Additional hormonal parameters and endometrial thickness were assessed. Hoogland scoring indicated ovulation in all women pretreatment, decreasing to 3 of 21, 1 of 23, 0 of 20, and 0 of 23 women in the 0.5-, 1-, 2-, and 3-mg groups, respectively (per-protocol set). Maximum serum estradiol concentrations were similar to pretreatment levels in the 0.5- or 1-mg group and decreased moderately (within physiologic levels) in the 2- or 3-mg group. Endometrial thickness was reduced by all dienogest doses. Hormonal changes during follow-up indicated resumption of ovulation in most women, shortly after treatment cessation. Dienogest ≥2 mg daily provides moderate suppression of estradiol production and reliable ovulation inhibition, which reverses rapidly after treatment cessation.

Keywords: dienogest; progestin; endometriosis; ovulation inhibition; contraception; estradiol

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Ovulation-inhibiting effects of dienogest

A previous small study in 8 healthy women reported that dienogest monotherapy demonstrates ovulation-inhibiting effects, assessed by changes in serum hormone concentrations, including progesterone, estradiol, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). For the current study, the ovulation-inhibiting effects of dienogest were reassessed in a large cohort of participants and included transvaginal ultrasound (TVU) assessment of the ovaries combined with serum hormone measurements. A demonstration of reliable ovulation inhibition by dienogest would offer potential benefits for women with endometriosis who wish to maintain contraception during treatment. Reversal of this ovulation inhibition shortly after treatment cessation would also offer advantages to women who wish to conceive after the end of therapy.

The aim of the current pharmacodynamic study in healthy volunteers was to compare the effects of clinically relevant doses of dienogest on ovulatory activity and other parameters relevant to contraception, using state-of-the-art assessment techniques. Study participants were followed up after cessation of dienogest treatment to assess their return to ovulation.

MATERIALS AND METHODS

Study Design

This was a single-center, randomized, double-blind, dose-controlled study of healthy female volunteers, conducted between September 2008 and September 2009.

Participants who fulfilled the study eligibility criteria were allocated by a computer-generated randomization list to receive 1 of 4 dienogest doses (0.5, 1, 2, or 3 mg daily), which were selected for their relevance to the clinical indications of dienogest.

Study periods included screening, washout, pre-treatment, randomization, treatment, and follow-up, as shown in Figure 1. Dienogest treatment was continued for a maximum of 72 days, with a treatment-free follow-up period of up to 72 days.

Figure 1. Study design. LH, luteinizing hormone.
Study Participants

Study inclusion criteria for women included age 18 to 35 years, a body mass index between 18 and 30 kg/m², ovulation demonstrated during the pretreatment period, and an interval of at least 3 months since delivery, abortion, or lactation. All participants were healthy with no clinically relevant medical conditions, vital sign abnormalities, or abnormal laboratory parameters.

Participants were required to use nonhormonal methods of contraception during the study. Pregnancy testing was performed at regular intervals using a commercially available β-human chorionic gonadotropin urine test. Study participation was to be terminated immediately in the event of a positive pregnancy test.

The study design was approved by an independent Ethics Committee (Stichting Beoordeling Ethiek Biomedisch Onderzoek, The Netherlands). Informed written consent was provided by all participants prior to study inclusion, in accordance with the Declaration of Helsinki. Each participant had the right to withdraw from the study at any time without providing a reason.

Study Medication

Study medication was provided to participants in 3 blister cards, each containing 28 tablets, with reserve cards available at the study center. Participants took 1 tablet orally each day for a maximum of 72 days. To maintain blinding, tablet formulations and packaging, regardless of dose, were identical in appearance. Participants were encouraged to take the study medication at the same time each day. Any missed tablets were taken as soon as remembered, unless more than 12 hours had elapsed since the normal time of intake, in which case the missed tablet was not taken; participants continued to take a tablet at the usual time on the next day. Participants were excluded from evaluation if they took less than 95% of the planned doses or if they missed pill intake on 2 consecutive days or on more than 2 days after treatment day 36. Treatment compliance was documented by the participants, who recorded the number of tablets taken and the time of tablet intake in diaries (which were inspected by investigators at each visit), and also by the return of blister cards, including any leftover pills at the end of the study.

Pharmacodynamic Measurements

Ovarian activity was assessed using the Hoogland scoring system, based on the diameter of the largest follicle-like structure (FLS) and serum estradiol (E2) and progesterone concentrations (Table I). Combining data on the growth pattern of FLSs with hormone measurement differentiates between cystic structures and the various stages of ovarian activity. The Hoogland score has been used in previous clinical trials to measure ovarian activity during COC. Hoogland scores were recorded for the pretreatment period and for 2 separate observation periods during treatment (days 1-36 and days 37-72).

TVU was performed at each study visit (approximately every 3 days) to examine both ovaries and the uterus. The largest FLS was identified and the diameter was calculated as the mean of the longitudinal and transverse diameters. Endometrial thickness was measured at the mediosagittal section (double layer).
Blood samples were taken at each visit during the pretreatment and treatment periods to determine serum E2, progesterone, FSH, and LH concentrations. Testing for an LH surge was also performed by participants at home on the morning urine using a digital ovulation test (Clearblue Digital, Unipath Ltd, Bedford, UK) during the pretreatment as well as the follow-up period until ovulation was confirmed.

The Insler score, a semi-quantitative assessment of the cervix and the amount and character of cervical mucus, was recorded after TVU if the FLS was >13 mm. Insler scoring ranges from 0 (hostile to conception) to 12 (concurrent with fertility/ovulation).

Safety Assessments

Adverse events (AEs) were classified according to Medical Dictionary for Regulatory Activities (MedDRA) coding. The intensity of AEs and their potential relationship to the study drug were assessed by the study investigators. Menstrual bleeding patterns were recorded by participants daily, on diary cards, with categorization as none, spotting, light, normal, or heavy.

Laboratory parameters assessed on blood samples or urine dipstick included hematology, liver function, and electrolytes (at screening, during pretreatment and treatment, and at final visit); HbA1c, thyrotropin, and virology (at screening); clotting status and urinalysis (at screening and final visit); and ferritin concentration (at screening and end of treatment). Blood pressure, heart rate, and body weight were recorded at screening, during pretreatment and treatment, and at final visit.

Statistical Methods and Analysis Sets

For continuous pharmacodynamic variables (ie, FLS diameter, endometrial thickness, and serum E2, progesterone, FSH, and LH concentrations), summary parameters (average and maximum) were calculated for the pretreatment and treatment periods. These summary parameters were analyzed using descriptive statistics and analysis of variance (ANOVA) to identify differences between dienogest doses. Categorical variables were analyzed using frequency tables.

The maximum Hoogland score over the 2 observation periods during treatment was summarized into 3 categories (ie, score 1 or 2, 3 or 4, and 5 or 6). This summarization was performed using the following rationale: a Hoogland score of 1 or 2 indicates no or minimum ovarian activity, a score of 3 or 4 may be characterized as residual ovarian activity, and scores of 5 and 6 indicate high ovarian activity, including ovulation. The dose-response relationship was assessed by a proportional odds model with a probit link function. Trichotomized Hoogland score was used as the ordinal response variable.

Statistical analyses were performed using Statistical Analysis Software (SAS) version 9.1.3 (SAS Institute, Inc, Cary, North Carolina). In view of the exploratory nature of this study, all P values and probability statements should be interpreted with caution. No measures for control of the overall level of significance were applied.

The sample size was determined so that (with a probability of 95%) the width of the 100 \( \times (1 - \alpha) \) prediction interval for ovulation at a dose of 2 mg dienogest would be \( \leq 0.3 \). Using \( \alpha = 0.05 \), a sample size of 20 volunteers per treatment arm was determined. Taking further into account a predicted 20% dropout rate, a minimum of 25 participants were planned to be recruited per treatment arm.

RESULTS

Study Participants

In total, 199 volunteers were screened, of whom 104 (52.3%) were randomized to study participation and 102 received treatment (full analysis set, FAS; Supplementary Figure S1). The FAS population had a mean age of 23 years (range, 18-35 years), height of 170 cm (range, 152-187 cm), and weight of 65 kg (range, 47-91 kg). Demographic characteristics were broadly similar across all dienogest dose groups.

No major protocol deviation was observed in 87 of the 104 randomized women (83.7%), and these women were included in the per-protocol set (PPS) (Supplementary Figure S1).

Ovarian Activity

Hoogland score. All women were classified as ovulating in the pretreatment period. During treatment with 0.5 or 1 mg dienogest (n = 21 and 23, respectively, in the PPS), 4 participants were classified as ovulating (n = 1, 0.5-mg group during the first observation period [days 1-36]; n = 2, 0.5-mg group during the second observation period [days 37-72]; and n = 1, 1-mg group during both periods). No women in the higher dose dienogest groups (2 and 3 mg; n = 20 and 23, respectively) ovulated during treatment in either observation period (Figure 2).
Figure 2. Ovarian activity (Hoogland score) in each dienogest dose group (% participants) for each observation period during treatment (per-protocol set [PPS]).

Probability of ovulation. The relationship between dienogest dose and trichotomized Hoogland score, assessed by a generalized linear model, is summarized in Supplementary Figure S2. For 2 mg dienogest, the probability of a Hoogland score of 5 or 6 (ie, luteinized unruptured follicle [LUF] or ovulation) was approximately 5% (95% confidence interval [CI], 0%-10%). For 0.5-mg and 1-mg doses, the predicted probability of a Hoogland score ≥5 was below 15%.

Follicle size. Dienogest had a significant dose-dependent effect on the mean (SD) of the largest FLS \( (P < .0001) \): 0.5 mg, 26.3 (14.8) mm; 1 mg, 21.4 (9.0) mm; 2 mg, 15.6 (5.4) mm; and 3 mg, 12.6 (4.1) mm during treatment \( (P < .0001, t \) test on differences between lower and higher dienogest doses).

Laboratory parameters. The dynamics of serum E2 concentrations during the pretreatment and treatment periods can best be shown in a concentration-time curve (Figure 3). During pretreatment, mean serum E2 concentrations increased from approximately 43 to 137 pg/mL. Individual maximum E2 concentrations during pretreatment reached a mean value of about 250 pg/mL (Table II) at around day 15.

During treatment with 0.5 mg and 1 mg dienogest, mean values of individual maximum E2 concentrations were 252 pg/mL and 212 pg/mL, respectively, similar to E2 concentrations during pretreatment (Table II). With 2 mg and 3 mg dienogest, the mean E2 concentration-time curve showed a moderate decrease of E2 from pretreatment values, with fairly stable values throughout treatment (see Figure 3). Mean concentrations of E2 during treatment were 39 pg/mL and 30 pg/mL with 2 mg and 3 mg dienogest, respectively, and the mean of the maximum concentrations was 79 pg/mL and 54 pg/mL (Table II). The change in average serum E2 concentrations during treatment supported the Hoogland score profile by showing substantial differences in effect between
lower (ie, 0.5 and 1 mg) and higher (ie, 2 and 3 mg) doses of dienogest ($P < .001$ for average as well as individual maximum E2 concentrations; $t$ tests on differences between lower and higher dose groups).

Serum progesterone concentrations during the pretreatment period reached a maximum at around day 24 (mean [SD] of the maximum concentration: 10.6 [4.0] ng/mL). The increase in progesterone concentration was suppressed by dienogest, with all serum values below 1.1 ng/mL for the 2-mg and 3-mg groups during all treatment days (data not shown).

The mean of the maximum FSH concentration during pretreatment was 9.9 mU/mL (Table II), which was attained, on average, on day 13. Dienogest had little overall effect on FSH concentrations, as the mean (SD) of the maximum value (9.3 [3.2] mU/mL, all dienogest doses) was similar to pretreatment values.

The mean of the maximum LH concentration was 28.5 mU/mL during pretreatment (Table II), which was reached, on average, on day 16. Dienogest decreased LH concentrations to a mean (SD) of the maximum value of 9.7 (3.5) mU/mL (all dienogest doses), with similar values in all 4 dose groups.

Endometrial Effects

Endometrial thickness was reduced by all dienogest doses (Figure 4). The mean of the maximum endometrial thickness was 10.0 mm during pretreatment and 6.6 mm during treatment (all dienogest doses). Mean endometrial thickness during treatment was similar in each dose group, with values of 4.0 to 4.5 mm at all time points. Even 0.5 mg dienogest produced a substantial suppression of endometrial growth.

Cervical Effects

During pretreatment, more than two-thirds of women had maximum Insler scores between 9 and 12, whereas approximately 25% had scores between 6 and 8, and the remainder (approximately 5%) had lower scores.

During treatment, Insler scores were measured in the 53 participants (60.9% of the PPS) who had a FLS >13 mm, predominantly in the 0.5-mg and 1-mg groups. The majority ($n = 38$; 43.7% of the PPS) had a maximum Insler score below 6, whereas the highest recorded score of 9 was measured in 2 women (2.3%; both in the 0.5-mg group).
Table II  Mean and Mean of the Maximum E2, FSH, and LH Concentrations During Pretreatment and Treatment (Per-Protocol Set)

<table>
<thead>
<tr>
<th>Assessment Period</th>
<th>Dienogest Dose Group</th>
<th>n</th>
<th>Mean ± SD(^a)</th>
<th>Mean ± SD(^b)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2, pg/mL</td>
<td>Pretreatment</td>
<td>Combined groups</td>
<td>87</td>
<td>116 ± 35</td>
<td>247 ± 84</td>
<td>84</td>
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<td></td>
<td>Treatment</td>
<td>0.5 mg</td>
<td>21</td>
<td>81 ± 53</td>
<td>252 ± 196</td>
<td>34</td>
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<tr>
<td></td>
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<td>1 mg</td>
<td>23</td>
<td>84 ± 71</td>
<td>212 ± 215</td>
<td>32</td>
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<tr>
<td></td>
<td></td>
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<tr>
<td></td>
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<td>3 mg</td>
<td>23</td>
<td>30 ± 8</td>
<td>54 ± 21</td>
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<tr>
<td></td>
<td>FSH, mU/mL</td>
<td>Pretreatment</td>
<td>Combined groups</td>
<td>87</td>
<td>5.2 ± 1.4</td>
<td>9.9 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
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<td>8.8 ± 2.4</td>
<td>4.7</td>
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<tr>
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<tr>
<td></td>
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<td>6.6 ± 1.1</td>
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<td></td>
<td>LH, mU/mL</td>
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<td>Combined groups</td>
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<td>28.5 ± 20.5</td>
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<td>Treatment</td>
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<td>5.5 ± 2.3</td>
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<tr>
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<td>5.4 ± 2.0</td>
<td>10.1 ± 3.8</td>
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<tr>
<td></td>
<td></td>
<td>2 mg</td>
<td>20</td>
<td>5.3 ± 2.1</td>
<td>9.0 ± 2.9</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 mg</td>
<td>23</td>
<td>5.8 ± 2.1</td>
<td>9.3 ± 3.2</td>
<td>4.5</td>
</tr>
</tbody>
</table>

\(^a\)P values of t test on differences between lower (ie, ≤ 1 mg) and higher (ie, ≥ 2 mg) dienogest doses (for individual average serum concentrations): 
E2: \(P < .001\); FSH: \(P = .001\); LH: \(P = .79\).

\(^b\)P values of t test on differences between lower (ie, ≤ 1 mg) and higher (ie, ≥ 2 mg) dienogest doses (for individual maximum serum concentrations): 
E2: \(P < .001\); FSH: \(P = .078\); LH: \(P = .196\).

Return to Ovulation

An LH surge in urine was identified in 60 of 87 women (69.0% overall; 0.5 mg: \(n = 18\) [85.7%]; 1 mg: \(n = 12\) [52.2%]; 2 mg: \(n = 16\) [80.0%]; 3 mg: \(n = 14\) [60.9%]) during follow-up, occurring between days 1 and 43 after cessation of treatment. Using the additional criterion of progesterone concentration above 1.6 ng/mL as an indicator of ovulation, only 2 women failed to resume ovulation during follow-up, whereas another 4 had missing data.

Safety Assessments

Dienogest was generally well tolerated during the study. Seventy-four women in the FAS reported an AE that was considered potentially treatment related. The most frequent treatment-related AEs (ie, ≥10% of participants in at least 1 dose group) included headache, dysmenorrhea, acne, nausea, lower abdominal pain, breast pain, gastrointestinal pain, and ovarian cyst (large FLS). Most AEs (91.8%) resolved during the course of the study. There was no clear relationship between the dienogest dose and the incidence or profile of AEs.

Two serious AEs were reported (ankle fracture and loss of consciousness), which were considered unrelated to treatment. Six AEs in 5 participants were associated with premature study withdrawal: 1 ankle fracture (mentioned above) and 5 AEs (metrorrhagia, headache, mood swings, and genital and pelvic pain) that were considered potentially treatment related.

Changes in laboratory values were infrequent, and none was attributed to the study medication. Ferritin deficiency (ie, <10 µg/L) was recorded in 6 (5.9%) women, whereas a slight elevation in liver enzymes (\(n = 3\)), increased bilirubin (\(n = 2\)), and altered hematology (\(n = 4\)) accounted for other abnormal laboratory results. A decrease in mean ferritin
concentrations of approximately 18% was seen over the study course, which was explained by the regular blood withdrawals totaling more than 250 mL. No relevant changes in vital signs or body weight were noted in any dienogest dose group.

Analysis of bleeding patterns was performed on women who completed treatment according to protocol. As expected, bleeding changed during treatment to a noncyclic pattern that is associated with the continuous administration of progestins. During the 72-day treatment period, the mean (SD) number of bleeding/spotting days was highest in the 1-mg dienogest group (24.2 [10.8] days) and lowest in the 2-mg group (18.1 [13.8] days). The intensity of bleeding/spotting decreased during continued treatment in all dose groups.

**DISCUSSION**

This study confirms that dienogest monotherapy possesses potent ovulation-inhibiting effects at all tested doses; complete ovulation inhibition was observed at doses ≥2 mg. Dienogest demonstrated a clear dose-dependent effect on most of the pharmacodynamic parameters studied, with a notable divergence between the 2 higher (2 and 3 mg) and the 2 lower (0.5 and 1 mg) doses. At 2 mg and 3 mg, Hoogland scoring demonstrated an inhibition of ovulation in all women. Modeling studies of the dose-response relationship confirmed the low likelihood of LUF or ovulation at these clinically relevant dienogest doses.

Recent clinical studies of dienogest have included 2 trial programs, performed in Europe and Japan, which investigated the efficacy and safety of this oral medication in endometriosis. These trials showed that dienogest, at the dose of 2 mg daily, offers an efficacy for pain relief that is statistically superior to placebo and equivalent to standard therapy with GnRH agonists. This efficacy profile is combined with favorable safety and tolerability over a treatment duration in excess of 1 year. Serum E2 concentrations were moderately reduced in these trials and tended to stabilize at the lower end of physiologic limits. Conforming with these observations, E2 concentrations in the current study showed a moderate decline at dienogest doses of 2 mg and 3 mg that were maintained within the physiologic

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**Figure 4.** Mean endometrial thickness at each visit during the pretreatment (combined groups) and treatment period (individual dose groups) (per-protocol set [PPS]). Error bars represent standard errors. Values at assessments are staggered for clarity.
range. The estrogen threshold hypothesis suggests that the optimal therapeutic window for endometriosis is achieved by a suppression of estrogen levels sufficient to inhibit endometrial stimulation but moderate enough to prevent hypoestrogenic side effects, such as bone mineral loss. It is notable that dienogest 2 mg daily was associated with stable lumbar bone mineral density (BMD) in a recent 24-week trial of women with endometriosis, whereas the comparator GnRH agonist (leuprolide acetate) induced a substantial decrease in BMD. A 1-year study of dienogest therapy in Japan reported no loss of BMD beyond that associated with normal aging. The absence of hypoestrogenic effects associated with dienogest distinguishes this therapy from GnRH agonists and certain other progestins, including depot medroxyprogesterone acetate, for which the US Food and Drug Administration has issued warnings concerning loss of BMD.

The changes in endometrial thickness in the current study indicated a potent endometrial effect for dienogest. All the doses investigated (including 0.5 mg) induced substantial suppression of endometrial growth. These observations, relating to the endometrial focus of dienogest, support preclinical and clinical studies that describe the efficacy of dienogest for inducing atrophy of endometriotic lesions. Together, these data show that 2 mg dienogest achieves a decrease in E2 concentration sufficient to reduce estrogen-dependent disease, combined with a systemic E2 exposure that minimizes hypoestrogenic side effects and likelihood of bone loss.

The current study demonstrated a prompt return to ovulation following cessation of dienogest treatment. These results confirm previous reports of a return to fertility after dienogest 2 mg daily, including treatment durations beyond a year. Early return to fertility is an important consideration for women who wish to conceive after successful endometriosis treatment, and this characteristic of dienogest is not shared by all other available medications.

A potential limitation of this study is the investigation of healthy volunteers rather than patients with endometriosis. However, a study of the ovulation-inhibiting effects of low dienogest doses (eg, 0.5 and 1 mg) would not be appropriate in patients with endometriosis, as the approved 2-mg daily dose has been shown to be optimal for pain relief. It may also be noted that no differences in ovarian function have been described between volunteers and patients with endometriosis.

The exclusion of women with obesity (body mass index >30 kg/m²) is a potential study limitation because of the possible impact of high body weight on the pharmacodynamic parameters investigated. However, more than 10 million women-years of experience with dienogest as a component of COC are available and suggest no impact of body weight on the contraceptive efficacy of this therapy.

In conclusion, oral dienogest monotherapy at a dose of 2 mg daily provides reliable suppression of ovulation, which reverses rapidly after treatment cessation. In addition, cervical factors as measured by the Insler score contribute to contraceptive activity. These data indicate that dienogest offers predictable contraceptive effects at the approved 2-mg daily dose.

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CK and ID are full-time employees and AR is a part-time employee of Dinox BV, Groningen, The Netherlands. TF, CZ, SK, and BS are full-time employees of Bayer Pharma AG, Berlin, Germany.

Authors’ roles: CK/ID/AR: Substantial contributions to conception, design, interpretation of data, and critical revision of the article. TF: Substantial contributions to interpretation of data and drafting and critical revision of article. CZ/BS: Substantial contributions to conception, design, interpretation of data, and drafting and critical revision of article.

REFERENCES
OVULATION-INHIBITING EFFECTS OF DIENOGEST


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