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Influence of combined oral contraceptive pills on the state of ovarian follicles and endometrium

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AUTHORS' CONTRIBUTION: **(A)** Study Design · **(B)** Data Collection · **(C)** Statistical Analysis · **(D)** Data Interpretation · **(E)** Manuscript Preparation · **(F)** Literature Search · **(G)** Funds Collection

SUMMARY

Introduction. Oral contraceptive pills are based on two fundamental compositional formulas: pills with estrogens and progestins as well as pills with progestins alone. The study was conducted to evaluate ovarian function during the use of oral contraception by comparing 5 products differing in the dose of ethinyloestradiol (EE) and the type of progestin.
Material and methods. It was a single-center, open-label, comparative study conducted in 89 healthy women divided into five groups: group 1: 0.03 mg of EE + 0.075 mg of gestodene (GSD), group 2: 0.03 mg of EE + 0.15 mg desogestrel (DGS), group 3: 0.02 mg of EE + 0.15 mg of DGS, group 4: 0.035 mg of EE + 0.25 mg of norgestimate (NGS), group 5: a triphasic preparation with 0.03–0.04 mg of EE + 0.05–0.125 mg of levonorgestrel (LNG). The 21+7 regimen was followed. On days 1–2 and 20–21 of the cycle, the number and diameter of ovarian follicles as well as endometrial thickness were measured with ultrasound. Moreover, estradiol and FSH concentrations were determined.
Results. There were no significant differences in estradiol levels between the groups. However, the mean FSH level on day 20–21 of the cycle in group 4 was significantly higher ($p = 0.006$) than the mean levels observed in groups 1, 2 and 5. The greatest number of ovarian follicles was found in group 5 (16.4), and the lowest in group 3 (12.6), but these differences were not statistically significant. Moreover, differences were also noticed in the diameter of follicles on day 1–2. The mean value in group 5 (4.6 mm) was significantly higher than in the remaining groups: 3.6 mm, 3.4 mm, 3.3 mm and 3.4 mm, respectively. The endometrium on day 20–21 of the cycle was the thickest in group 5 (7.2 mm) and was significantly thicker than in all the remaining groups ($p = 0.000005$).
Conclusions. All the tested products prevented ovulation in an effective way when used correctly. However, the level of follicular suppression and endometrial growth differed across the groups. Combined triphasic oral contraceptive pills with 30–40 μg of EE and 50–125 μg of LNG were characterized by lower suppressive capability than the products with lower EE doses. It can be suspected that the level of follicular suppression does not depend only on the EE dose, but also on the type of progestin, but the precise mechanism underlying this phenomenon remains unclear.
Key words: combined oral contraception; ovarian follicle; estradiol; follicle stimulating hormone

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INTRODUCTION

Oral contraceptive pills are based on two fundamental compositional formulas: pills with estrogens and progestins as well as pills with progestins alone. The most frequently used estrogen is ethinyloestradiol (EE) at a dose of 15–40 μg, and the progestins used include: levonorgestrel, desogestrel, drospirenone, gestodene, norgestimate or chlormadinone acetate.

The fundamental mechanism of action of combined oral contraceptive pill (COCP) is hypothalamic–pituitary–gonadal axis suppression, which decreases the level of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), and leads to reduced follicular activity, thus preventing ovulation. Moreover, COCP decreases sperm penetrability of the cervical mucus and alters the endometrium, thereby reducing sperm migration and lowering the probability of implantation [1]. Since its original development, COCP has been modified many times in order to reduce adverse events and increase acceptance with preserved contraceptive efficacy. When used correctly, the efficacy of pregnancy prevention is over 99%. The traditional regimen reflects the natural menstrual rhythm and envisages taking pills at the same time every day for 21 days with a 7-day hormone-free interval (HFI). Presently, the 24+4 regimen, which shortens the HFI, is becoming more and more popular. It has been proven, however, that reduced estrogen doses, which minimize adverse effects, may also decrease hypothalamic–pituitary–gonadal axis suppres-

sion, particularly during the HFI or when a pill has been missed, thereby causing increased ovarian activity [2].

The aim of this study was to assess ovarian function in patients using oral contraception by comparing 5 products with different EE doses and progestin types.

MATERIAL AND METHODS

Study

It was a single-center, open-label, comparative study conducted in the University Hospital of Obstetrics and Gynecology of Poznań University of Medical Sciences in Poland. It compared the effect of 5 different COCP products on the hormonal profile, endometrial thickness as well as the number and size of ovarian follicles in 3 consecutive cycles. The study was approved by the Ethics Committee of Poznań University of Medical Sciences.

Patients

The study included 89 healthy women aged from 18 to 45 years (mean age 27) with normal body mass index (mean BMI 23 kg/m2). The patients had regular menstrual cycles (26–34 days) without hyperandrogenism and contraindications to COCP. All patients had cervical smear conducted within the past 2 years. Prior to the inclusion in the study, all patients had a pelvic examination with a transvaginal ultrasound scan.

The study population was divided into five groups: group 1: 0.03 mg of EE + 0.075 mg of gestodene (GSD), group 2: 0.03 mg of EE + 0.15 mg desogestrel (DGS), group 3: 0.02 mg of EE + 0.15 mg of DGS, group 4: 0.035 mg of EE + 0.25 mg of norgestimatum (NGS), group 5: a triphasic preparation with 0.03–0.04 mg of EE + 0.05–0.125 mg of levonorgestrel (LNG). COCPs were taken at the same time of the day once daily from the 1st to 21st day of the cycle with a subsequent 7-day HFI.

Evaluated parameters

All patients were evaluated twice in each cycle: on days 1–2 and 20–21 of the cycle. Transvaginal ultrasound scans were conducted with Voluson 730 Expert machine using a 6 MHz endovaginal probe. All follicles in both ovaries were measured in two dimensions; their number and diameter were noted. Endometrial thickness was also measured. Moreover, the levels of 17ß-estradiol (E2) and FSH were determined by chemiluminescence using Elecsys 2010 analyzer by Roche Diagnostics. The sensitivity of the test for FSH is 0.1 mIU/mL, and for E_2: 5 pg/mL.

Statistical analysis

The values of the evaluated parameters were presented as arithmetic means plus standard deviation values (X ± SD). The statistical calculations were conducted with the equality of variance Brown–Forsythe test followed by ANOVA and *post-hoc* comparisons with the Newman-Keuls test. Values $p < 0.05$ were deemed statistically significant. The calculations were performed in STATISTICA by StatSoft Inc.

RESULTS

Patients were recruited between 2011 and 2015. Eighty-nine women were ultimately selected for the study. The analysis involved a total of 267 cycles with oral contraception. The highest estradiol levels on both days 1–2 and 20–21 were observed in groups 4 and 5, while the lowest were seen in groups 1 and 2. The differences were not statistically significant (Fig. 1 and Fig 2). As for FSH, its concentration on day 1–2 was lower in groups 1, 2 and 5 than in groups 3 and 4, but statistically significant levels were not reached either (Fig. 3). However, the mean FSH level on day 20–21 of the cycle in group 4 was significantly higher ($p = 0.006$) than mean levels observed in groups 1, 2 and 5 (Fig. 4).

Subsequently, the number of ovarian follicles was analyzed on day 1–2. The greatest number was found in group 5 (13.7), and the lowest in group 1 (12.2) with no statistical significance (Fig. 5). The results concerning the number of follicles on day 20–21 were different. Again, group 5 was characterized by the greatest number of follicles (16.4), while the lowest number was found in group 3 (12.6). Despite the evident trend suggesting greater ovarian activity in group 5, no statistical significance was achieved (Fig. 6).

Moreover, differences were also noticed on day 1–2 for another ovarian function parameter, i.e. the diameter of follicles. The mean value in group 5 (4.6 mm) was significantly higher than in the remaining groups: 3.6 mm, 3.4 mm, 3.3 mm and 3.4 mm, respectively (Fig. 7). Despite the fact that on day 20–21, the mean follicular diameter was the highest in group 5, the differences were not statistically significant (Fig. 8).

The last evaluated parameter was endometrial thickness with the greatest value measured on day 1–2 in group 5 (4.7 mm), but without a significant difference between other groups (Fig. 9). However, significant differences were noted on day 20–21, when the endometrium in group 5 (7.2 mm) was significantly thicker than in all the remaining groups ($p = 0.000005$) (Fig. 10). Ovulation was not observed in any of the patients. All COCP types were well-tolerated. There were no severe adverse events or any abnormalities in the clinical assessment. None of the patients withdrew from the study due to adverse events.

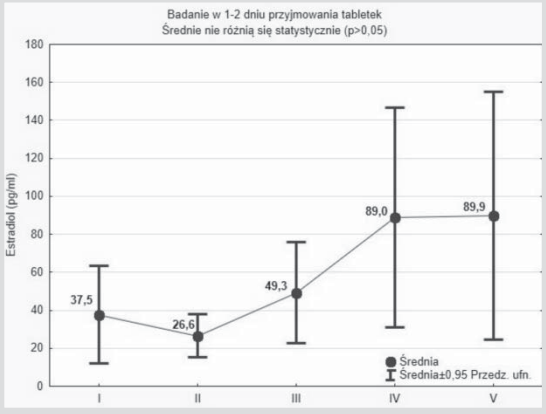

Fig. 1. Mean estradiol concentrations on day 1–2 across the groups

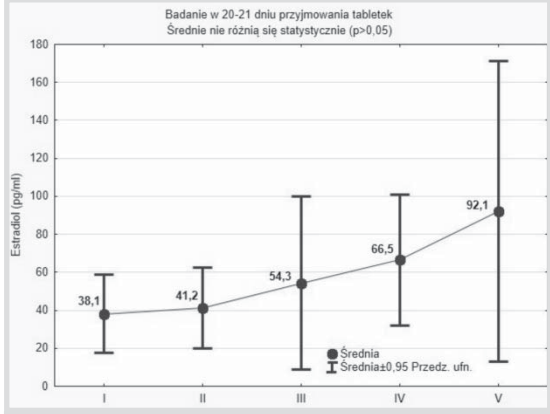

Fig. 2. Mean estradiol concentrations on day 20–21 across the groups

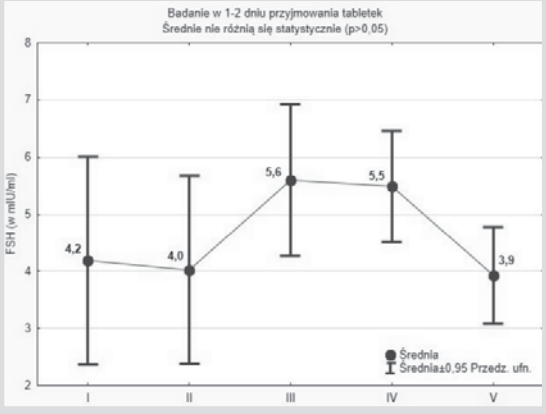

Fig. 3. Mean FSH concentrations on day 1–2 across the groups

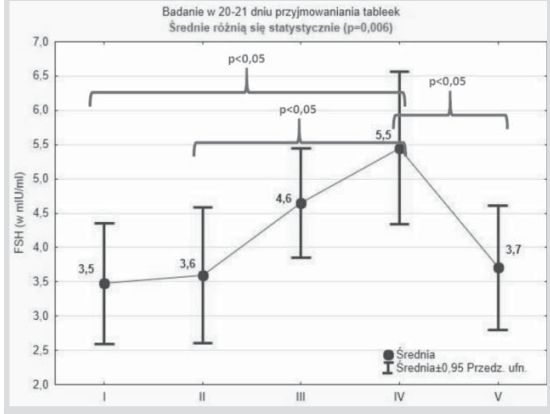

Fig. 4. Mean FSH concentrations on day 20–21 across the groups

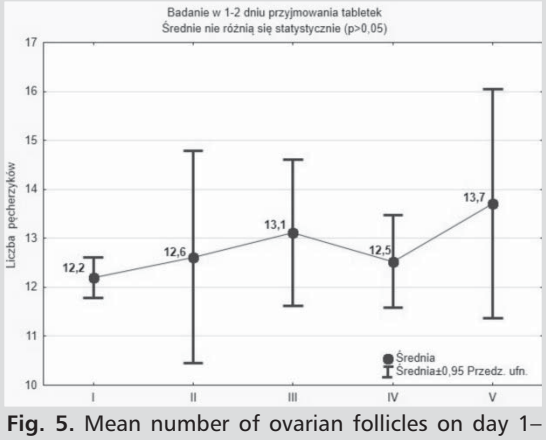

Fig. 5. Mean number of ovarian follicles on day 1–2 across the groups

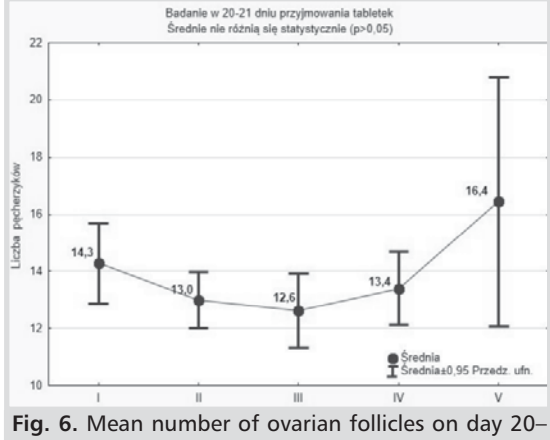

Fig. 6. Mean number of ovarian follicles on day 20–21 across the groups

DISCUSSION

Estrogens and progestins in supraphysiological concentrations decrease gonadoliberin (GnRH), FSH and LH secretion through negative feedback in the hypothalamic–pituitary axis, thereby suppressing follicular growth and ovulation [3]. By decreasing FSH secretion, estrogens suppress the growth of preantral follicles and antral follicles of average sizes, while progestins prevent the LH peak, thus blocking ovulation [4–6]. Additionally, estrogens improve patient comfort by preventing irregular uterine bleeding. It has been shown, however, that residual ovarian activity is preserved during hormonal contraception. Numerous authors believe that the grade of follicular suppression mainly depends on the dose of estrogens rather than on the type or dose of progestin [7,8]. Estrogen dose reduction decreases pituitary suppression, and increases follicular activity, particularly during the HFI and when a pill is missed [9]. Follicles ≥ 10 mm have been observed during the HFI in 86% of cases, while estradiol and FSH levels measured at the end of the HFI were comparable to those found in the early follicular phase [2]. If a dominant follicle does not develop during the HFI, follicular suppression continues. When, however, the dominant follicle does develop, its growth continues despite decreasing FSH levels [10]. It has been demonstrated that a shorter HFI (e.g. in 24+4 protocols) is characterized by greater suppressive action on both the ovaries and endometrium [11]. By contrast, the risk of failed contraception grows when the first or the last pill is missed and the HFI is prolonged [2,12]. Furthermore, it has been found that the grade of follicular growth during COCP use also depends on the length of natural cycles, the follicular phase in particular. Women whose follicles mature faster and ovulation occurs sooner have greater follicles also during COCP use than women with ovulation occurring later in the cycle. This phenomenon probably results from faster selection of the dominant follicle

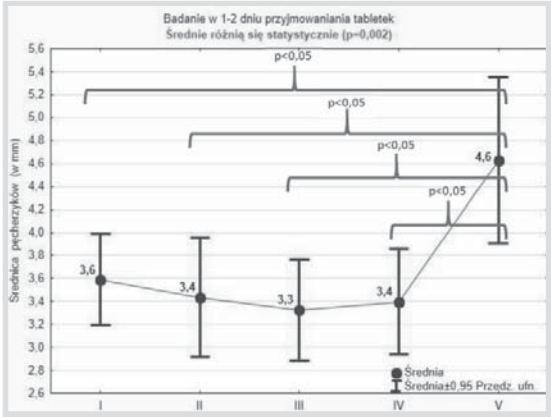

Fig. 7. Mean follicle diameter on day 1–2 across groups

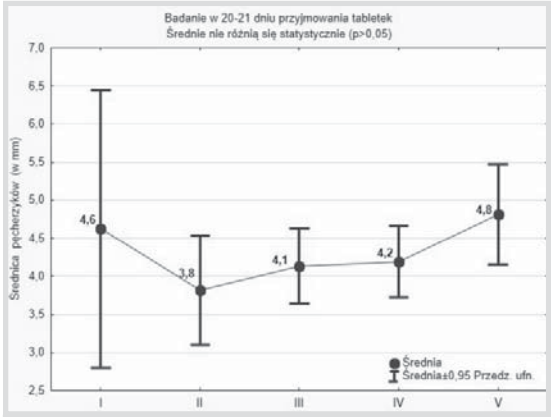

Fig. 8. Mean follicle diameter on day 20–21 across groups

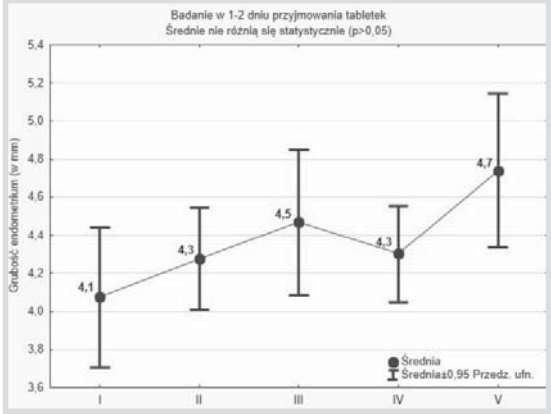

Fig. 9. Mean endometrial thickness on day 1–2 across the groups

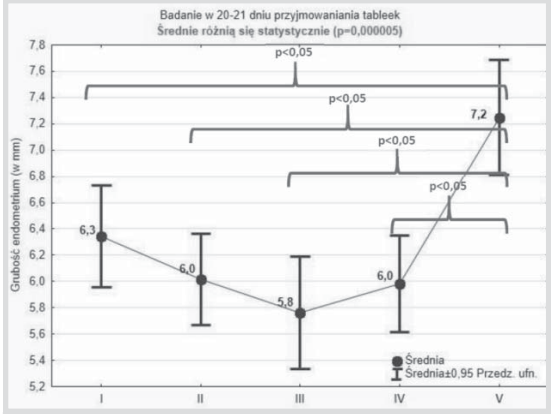

Fig. 10. Mean endometrial thickness on day 20–21 across the groups

during the HFI [13]. The impact of estrogens has also been shown in comparative studies. The maximum size and number of follicles during COCP use were greater in women using 20 μg of EE than in those using 30–35 μg of EE [14]. Moreover, a dose of 20 μg of EE has been associated with higher FSH and LH levels [8]. The risk of follicular growth and ovulation after unintentional missing of a pill increases for COC preparations containing 20 μg of EE compared to products with higher EE doses [15].

The hypothesis put forward by Fauser and Van Heusden, stating that estrogen doses are primarily responsible for the ovarian suppressive effect [7], has not been completely confirmed in the present study. The highest estradiol levels on both days 1–2 and 20–21 were observed in groups using the highest EE doses (group 4 and 5), but the differences were not statistically significant. After 20 days of using COCP, the highest number of follicles was found in group 5 (30–40 μg of EE), while the lowest in group 3 (20 μg of EE), but the differences were not statistically significant either. However, the mean size of follicles in group 5 on day 1–2 was significantly higher than in the remaining groups. Also, on days 20–21, the follicular diameter in group 5 was the highest, this time without statistical significance. Another finding worth emphasizing is a relatively high diameter of follicles (with a high standard deviation value) in group 1 with GSD compared to other third-generation progestins (DGS and NGS). Another parameter that reflects the grade of pituitary suppression by COCP is FSH concentration. It is an interesting observation that the highest concentrations were found in group 4 with a high EE dose (35 μg). These results suggest that it is the type of progestin that might have an impact on follicular development and hormonal ovarian activity. Similar doubts were mentioned in a different study comparing ovarian function during the use of COCP with 20 μg of EE and 1 mg of norethindrone acetate in the protocol with a 7-day HFI or a 4-day HFI [16]. By contrast with previous studies with similar designs but with different progestins, there were no differences between the two protocols.

Levonorgestrel was the first of the studied progestins. Later, in order to minimize the androgenic effects, gestodene, desogestrel and norgestimate were introduced [17]. The differences between the COCP products used in our study might results from several reasons. Third-generation progestins (GSD, DGS, NGS) are characterized by greater affinity to the progesterone receptor [18,19]. A minimum dose to suppress ovulation is 40 μg of GSD and 50 μg of LNG [20]. LNG is also characterized by the shortest half-life [18]. Another significant factor might be the impact of progestins on the hepatic synthesis of sex hormone-binding globulin (SHBG) and their related bioavailability. LNG doubles and GSD triples SHBG concentration [21]. SHBG levels in women using DSG are only slightly higher than in those using GSD [22]. The situation is further complicated by a variable level of progestin affinity to SHBG. GSD binds with SHBG in 75% while LNG in 47%. This might result in lower metabolic clearance and higher plasma concentration of GSD, which increases pituitary suppression and, in combination with longer half-life, improves contraceptive efficacy, e.g. in the case of missing a pill [23]. FSH suppression has been observed to be higher in patients using GSD compared with LNG [21].

The present study has also revealed significant differences in the action of the tested products on the endometrium. COCP has primarily progestagenic effects on the endometrium. Progestins decrease the endometrium-stimulating effect of estrogens, thereby inhibiting estrogen receptor expression [24]. On the first days of use, they induce secretory differentiation with coexistent proliferative and secretory features, and only several days later, the endometrium assumes the typical image of the "endometrium during contraception", i.e. atrophic glandular epithelium with tortuous glands similar to the secretory phase [25]. A study with 20 μg of EE and 2 mg of chlormadinone acetate revealed a decrease in endometrial thickness (mean ± SD) from 10.2 ± 3.0 mm (pretreatment cycles) to 5.1 ± 1.5 mm (cycle 1), 5.3 ± 2.1 mm (cycle 3) and 4.1 ± 2.2 mm (cycle 6) [26]. This could explain the thickness of the endometrium measured on day 20–21 in our study (5.8–7.2 mm) as our patients were followed for 3 cycles only. In our study, the triphasic pill containing 30–40 μg of EE and 50–125 μg of LNG was characterized by the weakest suppression of follicular activity and endometrial growth. Apart from progestagenic properties of LNG, this outcome could also be associated with a lower progestin dose for the first two weeks of use, compared to other monophasic products, and the highest dose of EE in the second week of use.

CONCLUSIONS

It must be concluded that all the tested products, when used correctly, prevented ovulation in an effective way. However, the level of follicular suppression and endometrial growth differed across the groups. Combined triphasic oral contraceptive pills with 30–40 μg of EE and 50–125 μg of LNG were characterized by lower suppressive capability than the products with lower EE doses. It might be suspected that the level of follicular suppression does not depend only on the EE dose, but also on the type of progestin, but the precise mechanism of this phenomenon remains unclear. Further investigations in follicular development during COCP use are needed to learn more about the mechanisms underlying hormonal contraception and influence of different types of progestins.

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