European Journal of Obstetrics & Gynecology and Reproductive Biology 164 (2012) 40–43

Contents lists available at SciVerse ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

The effect of ethinyl estradiol and drospirenone-containing oral contraceptives upon mucoprotein content of cervical mucus

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ARTICLE INFO

Article history:
Received 4 December 2011
Received in revised form 5 April 2012
Accepted 1 May 2012

Keywords:
Oral contraceptive
Cervical mucus
Cervical mucoprotein
MUC2

ABSTRACT

Objective: To report the effect of oral contraceptives (OC) on cervical mucoprotein content by evaluating quantitatively mucoprotein 1 (MUC1), mucoprotein 2 (MUC2), mucoprotein 5AC (MUC5AC) and mucoprotein 5B (MUC5B) levels.

Study design: This prospective controlled study included 20 women of reproductive age who had requested OC. Cervical mucus samples were obtained from the women before use of the OC and after 2 months of OC use. The mucus samples were then evaluated quantitatively for MUC1, MUC2, MUC5AC and MUC5B by ELISA by using specific antibodies.

Results: MUC5AC mucoprotein predominated quantitatively both before and after OC use. After OC use, compared to before OC use, variable increases in the levels of all studied mucoproteins were recorded, but the increases in MUC1, MUC2 and MUC5B were statistically significant. The difference in the level of MUC2 was remarkable (+54.36 ± 31.88 ng/mL).

Conclusion: OC use may change the mucoprotein content (especially for MUC2) of cervical mucus and thus, may cause a highly viscous pattern of cervical mucus which may enhance the contraceptive efficacy of OC pills.

1. Introduction

Hormonal contraceptives are female sex steroids containing synthetic estrogen and progestin or only progestin. The most commonly used hormonal contraceptives are combined oral contraceptives (OCs) [1]. With a high safety margin and other important health benefits, OCs today are the most effective reversible method of contraception, providing almost 100% effectiveness if used correctly [2]. The estrogen–progestin combination preparations consistently inhibit the midcycle gonadotropin surge and thus prevent ovulation. OCs also act on other aspects of the reproductive process. They alter the cervical mucus, making it thick, viscid and scanty, which retards sperm penetration [3]. The pathobiologic mechanism of this action, however, has not been well investigated.

Major structural components of mucus gel are mucoproteins which are composed of o-linked carbohydrates and highly glycosylated proteins. Mucins are secreted by the endocervical epithelium and protect the surfaces of the reproductive tract epithelium from pathogen penetrance as well as modulating sperm entry into the uterus [4]. At present, 20 types of mucoproteins have been defined. They are generally assessed in two categories: secreted mucoproteins and membrane bound mucoproteins. Secreted mucoproteins are also divided into two groups: gel forming and non-gel forming [5]. Among secreted mucoproteins MUC2, MUC5AC, MUC5B and MUC6, and among membrane bound mucoproteins MUC1 and MUC4 have been defined to be secreted by endocervix [4,6]. In a clinical study setting, the effect of oral contraceptives on cervical mucoprotein content has not been reported previously.

The aim of this clinical study is to report the effect of OCs on cervical mucoprotein content by evaluating quantitatively MUC1 (mucoprotein 1), MUC2 (mucoprotein 2), MUC5AC (mucoprotein 5AC) and MUC5B (mucoprotein 5B) levels.

2. Materials and methods

2.1. Study design

A total of 20 consecutive women requesting OC use for contraception at the Department of Obstetrics and Gynecology, Karadeniz Technical University were included in this prospective study. Institutional Review Board approval was obtained for the study. Each participant was provided with an informed consent

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http://dx.doi.org/10.1016/j.ejogrb.2012.05.002

form and signed that she understood the nature of the study and was willing to participate

2.2. Patient selection

The inclusion criteria were as follows: (a) willing to participate in the study, (b) of reproductive age (18–35 years), (c) outwith the pregnancy and lactation period, (d) no known systemic diseases (diabetes mellitus, hypertension, ischemic hearth disease, antiphospholipid Syndrome, acute or chronic renal disease, systemic lupus erythematosus, history of deep vein thrombosis or pulmonary embolism etc.) or cancer, (e) not a current smoker, (f) willing to use contraceptive pills, (g) no history of taking anticoagulant, non-steroidal anti-inflammatory, corticosteroid or other medications at least for two months, (h) having normal pelvic examination findings and cervical cytology results, (i) having normal renal, hepatic, and thyroid function test results, and (j) no history of contraception at least for two months. Patients not satisfying the inclusion criteria were not included to the study.

2.3. Study procedures

All women were subjected to pelvic examination on day 10 after the start of menses and cervical mucus samples (at least 0.5 mL) were collected and stored (as cervical mucus sample previous to OC use). On the next menstrual cycle the patients were instructed to start using an OC containing ethinyl estradiol (0.03 mg) and drospirenone (3 mg) (Yasmin® tablet, Schering-Bayer Farma, Istanbul, Turkey) on day 3 for three months. After completing two months of OC use, the women were recalled for pelvic re-examination on day 10 of the fourth menstrual cycle and cervical mucus samples (at least 0.5 mL) were collected and stored (as cervical mucus sample post-OC use). At the time of sampling for post-OC use, the women were still using the OC. In all cases, all samples were stored at −80 °C until analysis. Cervical mucus samples were then evaluated quantitatively for MUC1, MUC2, MUC5AC and MUC5B by ELISA by using specific antibodies.

2.4. Measurement of cervical mucoproteins (MUC1, MUC2, MUC5AC and MUC5B) on samples

Cervical mucus samples ($n = 40$) were evaluated quantitatively for MUC1 (Human Mucin 1, MUC1 ELISA Kit, Catalog number E0413h, USCNLIFETM, China), MUC2 (Human Mucin 2, MUC2 ELISA Kit, Catalog number E0705h, USCNLIFETM, China), MUC5B (Human Mucin-5 subtype B, MUC5B ELISA Kit, Catalog number E0684h, USCNLIFETM, China) and MUC5AC (Human Mucin-5 subtype AC, MUC5AC ELISA Kit, Catalog number E0756h, USCNLIFETM, China) by ELISA by using specific antibodies. The main test principle is given below.

The microtiter plates provided in for these kits have been pre-coated with an antibody specific to MUC1, MUC2, MUC5AC or MUC5B. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for MUC1, MUC2, MUC5AC or MUC5B. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain MUC1, MUC2, MUC5AC or MUC5B, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of MUC1 (ng/mL), MUC2 (ng/mL), MUC5AC (ng/mL) and MUC5B (ng/mL) in the samples is then determined by comparing the optic density of the samples to the standard curve.

The intra and interassay CVs for MUC1, MUC2, MUC5AC and MUC5B were below 8%.

2.5. Statistical analysis

Cervical mucoprotein levels were compared between the pre-OC use and post-OC use cervical mucus samples. Mucoprotein concentrations in each group were tested for normal distribution using the Kolmogorov–Smirnov test. Baseline measurements were compared using parametric (one-way ANOVA with Bonferroni correction as a post hoc test or Student's-*t* test) or non-parametric tests (Kruskal–Wallis test) when indicated. Intra-individual variations of antigens concentrations in the study group were assessed using the Paired Sample *t*-test. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS version 12.0, SPSS, USA). Statistical significance was set at $p < 0.05$. Data were expressed mean ± SD or number of cases and percentages in parentheses.

3. Results

The mean age of the women in the study group was 30.9 ± 4.4. The clinical characteristics of patients are summarized in Table 1.

The comparisons of mean cervical mucus MUC1, MUC2, MUC5AC and MUC5B levels between pre-OC and post-OC use samples are given in Figs. 1–4. The data illustrate the mean values for MUC1 (41.41 ± 1.76 ng/mL in pre-OC sample vs. 72.14 ± 16.99 ng/mL in post-OC sample, $p < 0.001$, paired sample *t*-test), MUC2 (37.94 ± 24.46 ng/mL in pre-OC sample vs. 92.30 ± 25.32 ng/mL in post-OC sample, $p < 0.001$, paired sample *t*-test), MUC5AC (89.68 ± 38.81 ng/mL in pre-OC sample vs. 110.45 ± 47.73 ng/mL in post-OC sample, $p = 0.055$, paired sample *t*-test) and MUC5B (42.90 ± 21.14 ng/mL in pre-OC sample vs. 95.91 ± 26.98 ng/mL in post-OC sample, $p < 0.001$, paired sample *t*-test) and show that these levels were significantly increased (except for MUC5AC) following two months of combined OC use.

Compared with other mucoproteins, MUC5AC mucoprotein levels predominated both before ($p < 0.001$, one-way ANOVA) and after ($p = 0.003$, one-way ANOVA) OC use, quantitatively. Compared to baseline levels, however, we noted a significant increase in the mean cervical MUC1 (+30.73 ± 24.44 ng/mL mean difference, 95% confidence interval (CI) 19.29–42.17, $p < 0.001$), MUC2 (+54.36 ± 31.88 ng/mL mean difference, 95% CI 39.44–69.29, $p < 0.001$) and MUC5B (+53.01 ± 33.68 ng/mL mean difference, 95% CI 37.25–68.78, $p < 0.001$) levels following two months of OC use. In contrast, a non-significant increase was observed in the mean cervical MUC5AC (+20.76 ± 45.38 ng/mL mean difference, 95% CI 0.47–42.00, $p = 0.055$). Comparison of mean increase in MUC1, MUC2, MUC5AC, and MUC5B levels from baseline revealed that the most significant increase in cervical mucoprotein levels was for MUC2 ($p = 0.005$, one-way ANOVA).

Table 1
Clinical characteristics of the patients.

Characteristics	Study group ($n = 20$)
Mean age (years)	30.9 ± 4.4
Mean gravida (number)	2.5 ± 1.2
Mean parity (number)	1.8 ± 1.1
Mean body-mass index (kg/m^2)	22.9 ± 2.6
Mean menstrual cycle length (day)	26.6 ± 3.7
Mean age at marriage (years)	19.8 ± 2.6
Previous history of contraceptive method use (%)	
Absent	10 (50%)
Present (oral contraceptives)	5 (25%)
Present (intrauterine device)	5 (25%)

Data are expressed as mean ± standard deviation, number of patients and percentages in parantheses.

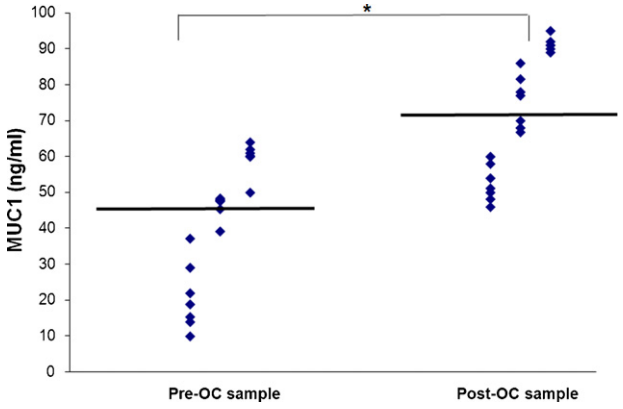

Fig. 1. The comparison of mean cervical mucus MUC1 (ng/mL) levels between pre-OC and post-OC use samples (*$p < 0.001$, paired sample-t test).

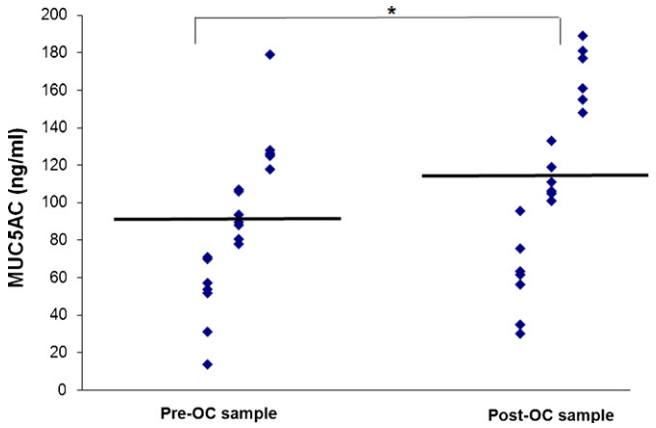

Fig. 3. The comparison of mean cervical mucus MUC5AC (ng/mL) levels between pre-OC and post-OC use samples (*$p = 0.055$, paired sample-t test).

During the study period there were no OC-related side or adverse effects.

4. Comment

In our study, we found that following two months of OC use, all analysed mucoprotein (MUC1, MUC2, MUC5AC and MUC5B) levels increased, but a statistically remarkable increase was determined for one of the gel-forming mucoproteins, MUC2.

During a normal menstrual cycle, but outside the ovulatory phase, the mucus is scant, thick and viscous, thereby forming a physical barrier that limits the access of sperm to the genital tract. In contrast, immediately before ovulation cervical mucus viscosity decreases, thereby maximizing its permeability to sperm [7,8]. It has been believed that this function of human cervix is achieved by mucoproteins which are secreted by endocervical cells. Steroid hormones strongly influence mucin biosynthesis in endocervical cells, and mucin 5B (MUC5B) becomes the principal mucin present in human ovulatory cervical mucus [4,9,10]. In a recent study, however, it was reported that MUC5AC mucin was also present in substantial amounts in human cervical mucus. Proteomics analysis of human cervical mucus suggested that all analyzed mucin bands contain mixtures of mucins, and MUC5B and MUC5AC peptides were found in all analyzed bands [11]. Our study findings are well correlated with this study, since we also reported that the major cervical mucoproteins were MUC5B and MUC5AC in pre-OC use cervical mucus samples.

In one study observing the change of mucoprotein content in cervical mucus at certain periods of menstrual cycle, the authors defined relative abundance of mucoproteins in cervical mucus. One of the gel-forming mucoproteins, MUC2, was represented with faint change levels in two samples [6]. In our study, both before and after OC use, one of the gel-forming mucoproteins, MUC5AC, was shown as the dominant mucoprotein in cervical mucus. Following MUC5AC, with respect to their amount in cervical mucus, MUC2, MUC5B and MUC1 dominated, respectively. This ranking of mucoproteins did not change in the evaluation of cervical mucus samples obtained after OC use.

We know that viscosity of cervical mucus increases due to domination of progesterone throughout the second half of menstrual cycle. This increase in viscosity does not allow sperm penetration. Because of their progestin content, OCs also cause the same effect and this contributes to contraception. It is unclear which mucoprotein content change causes this effect in cervical mucus. The amount of MUC5B mucin in human cervical mucus varies during the menstrual cycle and peaks at ovulation, suggesting that this larger mucin species may be an important factor affecting sperm transit to the uterus [4,10]. It has been suggested that MUC5B, owing to its hydrophilic structure, unexpectedly reduces the viscosity by keeping water so as to enhance sperm permeability through endocervix [10]. It was expected that OC may change the MUC5B mucin levels in human cervical mucus in order to enhance its contraceptive efficacy and change the cervical mucus from thin to thick pattern.

Accordingly, in our study, in cervical mucus samples obtained after OC use, when compared with those obtained before, an increase in the amounts of all four mucoproteins was examined,

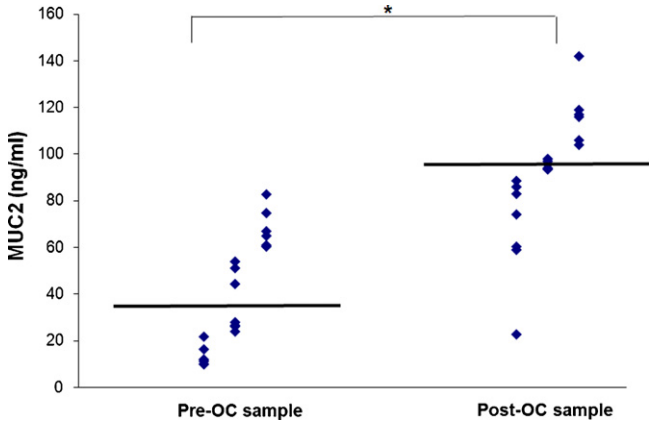

Fig. 2. The comparison of mean cervical mucus MUC2 (ng/mL) levels between pre-OC and post-OC use samples (*$p < 0.001$, paired sample-t test).

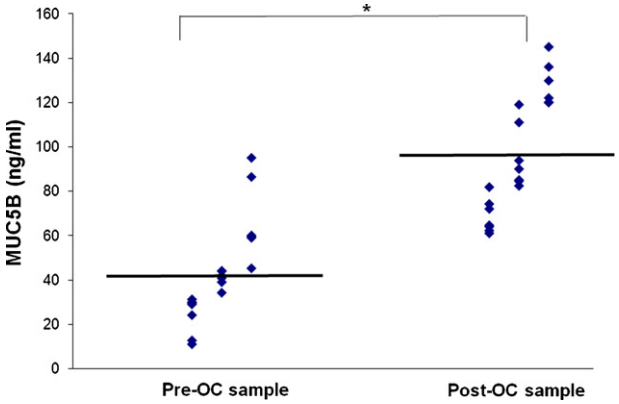

Fig. 4. The comparison of mean cervical mucus MUC5B (ng/mL) levels between pre-OC and post-OC use samples (*$p < 0.001$, paired sample-t test).

and the increase in three of them (MUC1, MUC2, MUC5B) was statistically significant. On the other hand, according to Gipson et al's findings [6], in midcycle when cervical mucus is thin and watery, quantitative amounts of MUC5B and mRNA levels of MUC4 were high, and those values, having been inconsistent with our study, reduced at the luteal phase of cycle when progesterone dominated. Interpreting both studies, but assuming according to Gipson et al's findings that MUC5B levels reduce in the luteal phase of the cycle, MUC2 is likely to be the most important mucoprotein responsible for the thickening process of cervical mucus. Although due to our study, a significant increase in MUC5B levels with OC use was shown, this increase was not as notable as that of MUC2 and this increase might not be solely responsible for the process of cervical mucus becoming viscous and scanty. The increase in MUC2 levels may be responsible for the contraceptive efficacy of cervical mucus. More large-scale studies are needed on the functions of specific mucoproteins in the cervix and their quantitative amounts at different phases of the menstrual cycle.

MUC2 expression has been reported to be involved in gastric, pancreatic and colorectal cancer carcinogenesis [12–14]. Baker et al. [15] reported that MUC1, MUC4 and MUC5AC were expressed by all benign endocervical epithelia, while MUC2 was not. The MUC2 might be expressed more by neoplastic lesions or this mucoprotein might cause a tendency for neoplasia in cervical epithelium in which it is expressed more [15–17]. In our study, in the mucus samples obtained after OC use, when compared to those obtained before, a statistically remarkable increase was recorded for MUC2 mucoprotein. This result should let us consider the idea that cervical mucus MUC2 mucoprotein increase may contribute to cervical carcinogenesis in OC users, and indicates that studies are needed in wide-ranging populations to determine the causal connection of the relationship between MUC2 and cervical carcinogenesis [18].

In conclusion, we believe that the increase in MUC2 level may be the main element responsible for the viscous and scanty structure of cervical mucus. This also contributes to the contraceptive efficacy of combined oral contraceptives. We also speculate that the OC-induced MUC2 increase in cervical mucus may be the underlying mechanism of OC-induced cervical carcinogenesis, although large-scale longitidunal studies are needed.

Conflict of interest

None declared.

Acknowledgment

We thank Karadeniz Technic University, Scientific Research Project Unit for their funding support (Project number 2008.114.002.8).

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