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Differences in sex hormone levels in the menstrual cycle due to tobacco smoking — myth or reality?

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Abstract

Introduction: Tobacco smoke contains, among others, polycyclic aromatic hydrocarbons (PAHs), heterocyclic analogues, aromatic amines, N-nitrosamines, volatile hydrocarbons, aldehydes, phenols, miscellaneous organic compounds, metals, and inorganic compounds. Tobacco smoking can harm women's reproductive system and may reduce fertility. The objective of the study was to explore the effect of tobacco smoke on the menstrual cycle due to smoking and second-hand smoke-exposure.

Material and methods: The study was performed on 153 women of reproductive age, who received care at the Gynaecological-Obstetric Clinical Hospital of the Poznan University of Medical Sciences. They were divided into three treatment groups: non-smokers, second-hand smokers, and smokers.

Comprehensive assessment of all hormone levels: follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17β-oestradiol (E2), and progesterone (P), in the various phases of the menstrual cycle and with concomitant determinations of serum cotinine concentrations was performed. The menstrual cycle was observed with ultrasonography.

Results: Cigarette smoking may be an important factor in disrupting reproduction: 1. The increase in the oestradiol E2 level was accompanied by significantly lowered serum cotinine concentrations in tobacco smokers; 2. In smoking patients, the serum level of LH significantly increased on the first days of the menstrual cycle; 3. The higher levels of P (in the 14th and 21st days) were assumed to be the result of a longer menstrual cycle.

Conclusions: Active and passive smoking may be an important contributor to reproductive health issues and deserves greater focus in health education programs directed towards women of reproductive age. **(Endokrynol Pol 2022; 73 (1): 16–25)**

Key words: *menstrual cycle; tobacco smoking; sex hormones; fertility*

Introduction

Tobacco smoke constitutes, among others, polycyclic aromatic hydrocarbons (PAHs), heterocyclic analogues, aromatic amines, N-nitrosamines, volatile hydrocarbons, aldehydes, phenols, miscellaneous organic compounds, metals, and inorganic compounds [1–4]. Smoking is one of the most serious hazards to modern civilization because of its widespread use, the effect on human health and life, and the monetary losses experienced by society [5]. Of great concern was the adverse effect that tobacco smoking demonstrated in both clinical and epidemiological investigations in women of reproductive age [6–10]. According to World Health Organization (WHO) data, currently 175 million smokers in the population are women, whereas only in Europe, there are 74 million [5, 11–12]. In Poland, there was a dramatic increase in tobacco smoking among young girls (57% are smokers). Recent meta-analysis by Vardavas and Nikitara, and another by Cai showed that tobacco use is associated with adverse outcomes and a worse prognosis for those with Coronavirus Disease 2019 (COVID-19) [13, 14].

Nicotine and its main metabolite, cotinine, negatively affect gametogenesis, oviduct transport, ovulation, fertilization, implantation of the fertilized oocyte, as well as the development of the placenta. Tobacco smoking and exposure to second-hand smoke result

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in a lower expression of the gene coding for the receptors of follicle-stimulating hormone (FSH) and causes a decrease in levels of this hormone produced by granulosa cells. Inactivation of aromatase by the alkaloids in tobacco smoke results in decreased production of 17β-oestradiol (E2). Polycyclic aromatic hydrocarbons present in tobacco smoke induce modifications in the action of microsomal enzymes, the cytochrome P450, CYP19, and inhibition of aromatase in granulosa cells [10, 15–16].

Since the 1980s there have been many meta-analyses and cohort studies on the relationship between the menstrual cycle and changes in the number of smoked cigarettes [17–21]. These studies showed an increase in the number of smoked cigarettes in the luteal phase. In smokers, significantly reduced levels of anti-Müllerian hormone (AMH) were found, especially in patients preparing for in vitro fertilization (IVF) [22–25]. The latest research by Lammert et al. showed that women who use tobacco and cannabis have a shortened luteal phase in comparison to females who use tobacco only [26]. Some studies have reported that women smoke more cigarettes per day during the luteal phase of the cycle [27], although not all studies report this [28]. Furthermore, abstaining from smoking during the premenstrual phase appears to increase the discomfort associated with quitting compared with abstaining in other phases.

The goal of the present study was to analyse the influence of exposure to tobacco smoke (active and passive smoking) on the level of luteinizing hormone (LH), FSH, 17β-E2, and P in blood serum of young Polish women in the 1st, 14th, and 21st day of the menstrual cycle.

Material and methods

Patients and sample collection

The study was performed on a group of 153 patients in reproductive age (range 17.4–24.6 years) (Tab. 1), who received care at the Gynaecological — Obstetric Clinical Hospital of Poznan University of Medical Sciences, Poland. The authors obtained informed consent from the participating women. The research project received consent by the Bioethical Commission of Poznan University of Medical Sciences, Poland.

The patients were a group of healthy volunteers, who were invited by the hospital for prophylactic gynaecological examination with contemporary hormonal analysis, as well as estimation of carcinoma antigen 125 (CA-125), carcinoembryonic antigen (CEA), and alpha-fetoprotein (AFP). The age group was uniform.

They were divided into three treatment groups: non-smokers (n = 42), second-hand smokers (n = 49), and smokers (n = 62). The assignment to each research group was made on the basis of each patient's report of their smoking status (basis for assignment to the appropriate group) – and as an auxiliary indicator, the cotinine level in serum. The ovulatory cycle was confirmed by vaginal ultrasound in each case [29].

Women with diabetes, primary ovarian insufficiency, depression, anxiety, thyroid disorders, asthma, allergies, seizures, and migraines as well as with transvaginal ultrasound abnormalities, smoking e-cigarettes, taking illegal drugs, nicotine replacement preparations, and hormonal contraceptives drugs were excluded from the study.

Transvaginal ultrasound assessment was performed on days 12–13 and 15–16 in the whole group, to determine the phase of the cycle. The goal was to find the dominant follicle collapse as the indirect sign of ovulation. The duration of the menstrual cycles ranged from 24 to 39 days, with the luteal phase from 9 to 16 days, according to TVU evaluations (Tab. 1).

The blood samples were collected (around 8 a.m.) from test participants in the 2nd or 3rd, 14th, and 21st day of the menstrual cycle (three samples were analysed from one subject). After 10 minutes

Table 1. *Clinical characteristics of study participants*

	Non-smokers (n = 42)	Second-hand smokers (n = 49)	Smokers (n = 62)
Age [years] mean (±SD)	22.1 (2.1)	21.7 (2.3)	19.2 (1.9)
Range	19.1–24.6	18.2–23.7	17.4–23.2
Number of cigarettes per day, mean (±SD)	0	0	16.4 (4.7)
FTND Score, mean (±SD)	–	–	5.3 (1.9)
BMI [kg/m^2] mean (±SD)	23.5 (1.9)	22.4 (2.8)	23.7 (2.6)
Menarche age (mean)	12.8	12.6	13.2
Level of serum cotinine [ng/mL]*			
Mean ± SD (n)		30 ± 19.1 (13)	96.6 ± 120.8 (91)
In 2–3/14/21 day	0	26.23/34.27/32.96	110.72/78.21/99.25
Duration of menstrual cycle			
Mean (±SD)	31.5 (2.5)	30.1 (2.4)	29.7 (2.2)
Range	25–38	26–39	24–38
Duration of luteal phase (days) mean (±SD)	11.5 (1.7)	11.2 (1.4)	10.1 (1.2)
Range	10–16	10–15	9–14

*The cotinine level should be interpreted with caution because in the second-hand smokers group there were only 9% and in the smokers group 45% of the results above the lower limit of quantification (LOQ); FTND — Fagerström Test for Nicotine Dependence; BMI — body mass index; SD — standard deviation

of centrifugation at 2000 g at 4°C, the serum was aspirated and frozen at –80ºC for storage for later measurement (not longer than one month).

Questionnaire and Fagerström Test for Nicotine Dependence (FTND)

Each woman who participated in the study was asked to fill out a questionnaire to assess socio-economic status, health conditions, exposure to tobacco smoke, and the course of her menstrual cycle. Information collected from the questionnaire protocol, following appropriate coding, was subjected to statistical analysis. The Fagerström Test for Nicotine Dependence was used as a standard instrument to assess the intensity of physical addiction to nicotine (Tab. 1) [30].

Serum cotinine determination

Cotinine levels were determined by high-performance liquid chromatography with diode array detection (HPLC-DAD). The internal standard was norephedrine — 100 μL/mL. Chromatography was preceded by a liquid–liquid extraction. The sodium hydroxide solution (0.2 mL of 0.1 M) was added to 1 mL of serum to obtain pH = 8. Then 150 μL of norephedrine was added. Liquid–liquid type extraction from the prepared samples was performed. For liquid–liquid extraction, a mixture of dichloromethane and isopropanol at 9:1 (v/v) was added to the samples. Next, samples were shaken for 15 min (Multi Bio RS-24 shaker — Biosan). Then samples were centrifuged for 15 min at 3100 g (Centrifuge 5804/5804 R, Eppendorf). To the obtained extract 4 mL, with 150 μL of hydrochloric acid 0.035 M in methanol was added, and evaporated under pressurized nitrogen. The dry residue was dissolved in 100 μL of mobile phase [31]. Qualitative and quantitative analyses were performed using liquid chromatography by Agilent Technologies series 1200 with a diode array detector (Perlan Technologies). A liquid chromatography column with C8 silicone filling: 5 μm, 125 × 4 mm and precolumn: 5 μm, 4 × 4 mm (LiChrospher 60 RP-Select B by Merck) were used. The mobile phase was a mixture of acetonitrile-phosphate buffer with pH 4.2 (10:90) and flow rate of 1.0 mL/min. The injection volume was 50 μL. The absorbance was measured at a wavelength of 260 nm.

Before starting the studies, the method was subjected to validation, with such validation parameters as follows: limit of detection (LOD), limit of quantification (LOQ — 15 ng/mL), linearity (15–1000 ng/mL), and reproducibility within a day (2.10% and 7.34%) and between days (3.76% and 8.41%). The certified reference material was used to optimize and validate the method [31].

Sex hormones analysis

In serum collected from all participants, the following hormones FSH, LH, E2, and P were measured by commercially available kits in accordance with the instructions of the manufacturer. The measurements were made using electrochemiluminescence immunoassay — ECLIA (Modular Analytics, E170; Roche Diagnostics, Mannheim, Germany).

The respective intra- and interassay coefficients of variations were as follows: FSH 1.3–2.8 and 3.6–4.5%, LH 0.6–1.2 and 1.6–2.2%, E2 1.4–3.3 and 2.2–4.9%, and P 0.7–2.9 and 1.9–4.9%. LOD were as follows: FSH 0.1 mIU/mL LH 0.1 mIU/mL, E2 5.0 pg/mL and P 0.03 ng/mL. Normal values in our laboratory are as follows: FSH (mIU/mL): follicular phase from 3.5 to 12.0; ovulatory phase from 4.7 to 21.5; luteal phase up to 1.5; LH follicular phase from 2.5 to 12.5 mIU/mL; ovulatory phase from 14. 0 to 95.6; luteal phase from 1.0 to 11.5; E2 (ng/mL): follicular phase from 12 to 233; ovulatory phase from 41 to 398; luteal phase from 22 to 341. Progesterone (ng/mL): follicular phase from 0.057 to 0.893; ovulatory phase from 0.121 to 12.0; luteal phase from 1.83 to 23.9.

Statistical analysis

SAS 9.4 software (SAS Institute Inc., Cary, NC, USA) was used to perform statistical analysis. The normality of hormone concentration data was checked with Shapiro-Wilk test. Based on the results of the Kruskal-Wallis test, non-parametric analysis was implemented. When necessary, it was subsequently followed by pairwise two-sided multiple comparison analysis among subject groups using Dwass, Steel, Critchlow-Fligner method. The differences were considered significant for $p < 0.05$.

Results

Cotinine levels

Appropriate assignment of women to their active smoking, second-hand smoke exposure, or non-smoking groups were corroborated by using cotinine levels in the serum of the studied women on the 2–3rd, 14th, and 21st day of the menstrual cycle that was partly concordant with the histories provided by these women. However, following our analysis, 13 women from the non-smoking group moved to the smokers' group because of high nicotine metabolite levels in their serum.

In women exposed to second-hand smoke and in active smokers, the mean concentration of cotinine in the serum was 26.23, 34.27, and 32.96 ng/mL and 110.72, 78.21, and 99.25 ng/mL on the 1st, 14th and 21st day of the menstrual cycle, respectively (Tab. 1). The cotinine concentrations between these groups were significantly different ($p < 0.0001$).

Based on the Fagerström Test for Nicotine Dependence (FTND), all patients who smoked, showed a statistically significant degree of nicotine dependence (over 6 points) (Tab. 1).

Sex hormones levels

Non-smoking

In this group, normal fluctuations were noted in the levels of FSH, typical for menstrual cycles. The level of FSH on the 1st day was higher (5.37 mIU/mL) than on the 14th and 21st days of the cycle (4.68 mIU/mL and 4.16 mIU/mL, respectively) (Fig. 1).

Also, levels of LH in the control group changed in the normal manner. There was a low concentration of LH on the 1st day of the menstrual cycle (4.80 mIU/mL); the highest was on the 14th day (8.92 mIU/mL) and then it decreased on the 21st day (6.02 mIU/mL) (Fig. 2).

The concentrations of E2 in serum pointed to normal levels in the menstrual cycle. They amounted to 39.89 pg/mL, 108.60 pg/mL, and 109.20 pg/mL on the 1st, 14th, and 21st day, respectively (Fig. 3).

Low levels of P (0.61 ng/mL) were seen on the 1st day, they increased on the 14th day (0.96 ng/mL), and reached the highest value (1.52 ng/mL) on the 21st day of the menstrual cycle (Fig. 4).

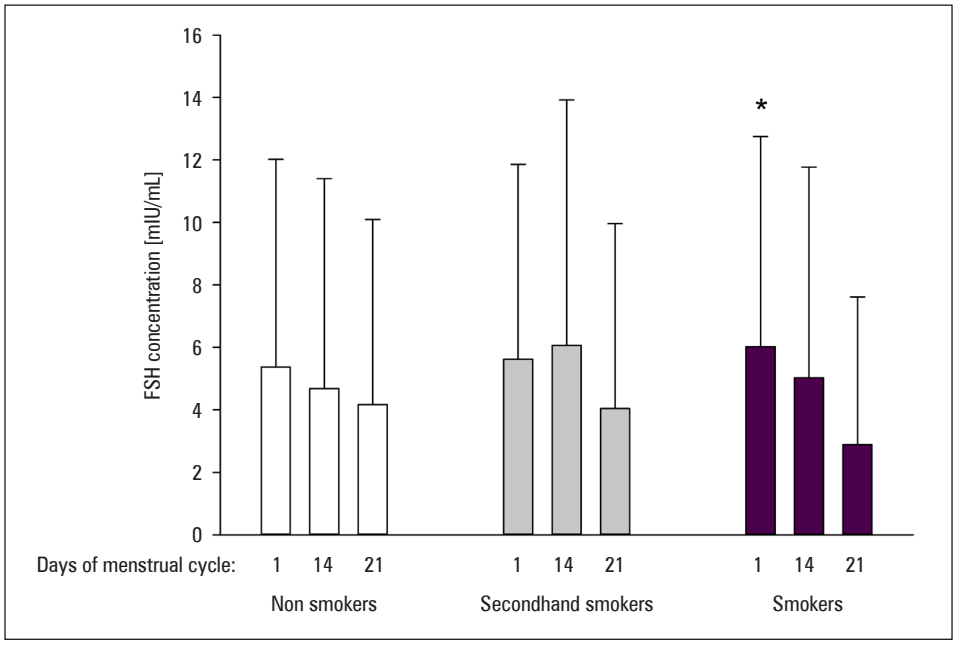

Figure 1. *Mean serum concentrations of folliculotropic hormone (FSH) concentrations in the groups of non-smoking, exposed to second-hand smoke, and smoking women in the 1st, 14th, and 21st day of the menstrual cycle. *Statistically significant difference between smokers and second-hand smokers (p = 0.02)*

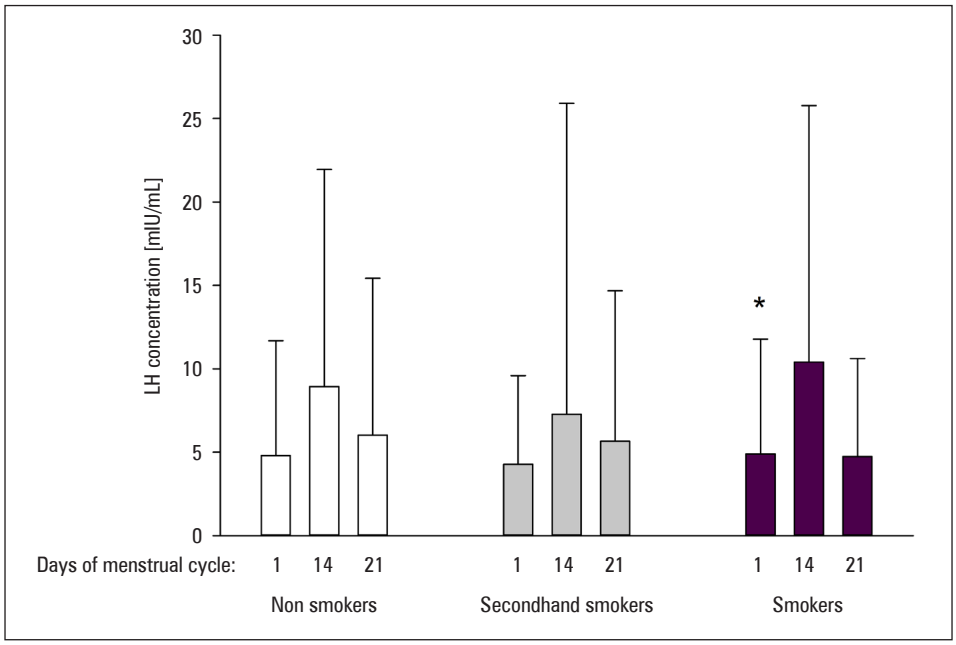

Figure 2. *Mean serum concentrations of luteinizing hormone (LH) in the groups of non-smoking, exposed to second-hand smoke, and smoking women in the 1st, 14th, and 21st day of the menstrual cycle. *Statistically significant difference between smokers and second-hand smokers (p = 0.03)*

Second-hand smokers

In this group, the levels of FSH on the 1st, 14th, and 21st day of the menstrual cycle were 5.62 mIU/mL, 6.06 mIU/mL, and 4.04 mIU/mL, respectively (Fig. 1). The LH level detected on the 1st day of the menstrual cycle was 4.27 mIU/mL, then it increased on the 14th day (7.27 mIU/mL), and it decreased again on the 21st day (5.65 mIU/mL). These alterations in hormone levels were similar to those of non-smoking women (Fig. 2). The concentration of E2 indicated a normal level during the menstrual cycle and amounted to 34.88 pg/mL, 118.20 pg/mL, and 110.10 pg/mL, respectively

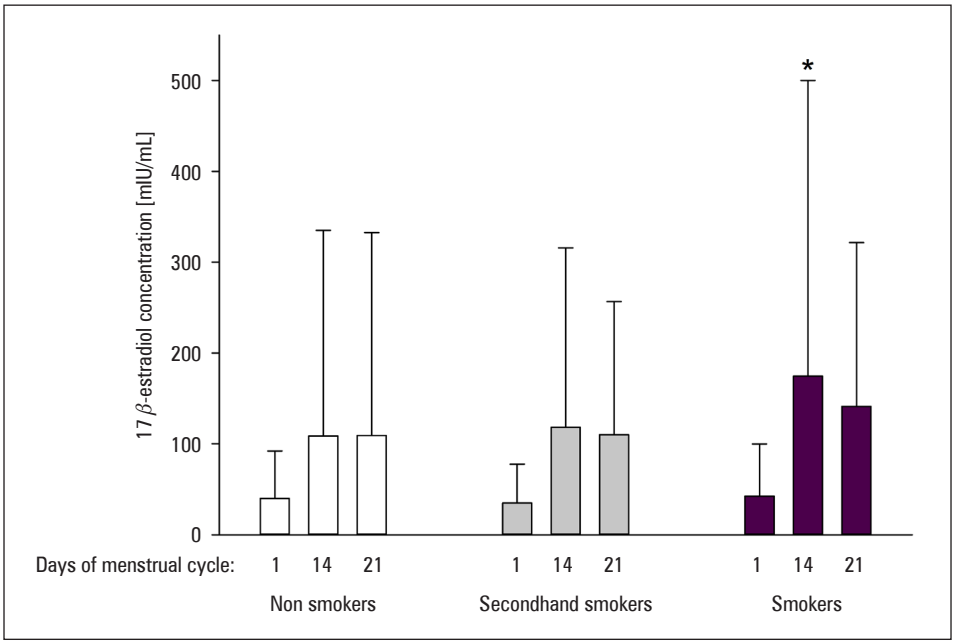

Figure 3. *Mean serum concentrations of 17β-oestradiol (E2) in the groups of non-smoking, exposed to second-hand smoke, and smoking women in the 1st, 14th, and 21st day of the menstrual cycle. *Statistically significant difference between smokers and second-hand smokers (p = 0.02)*

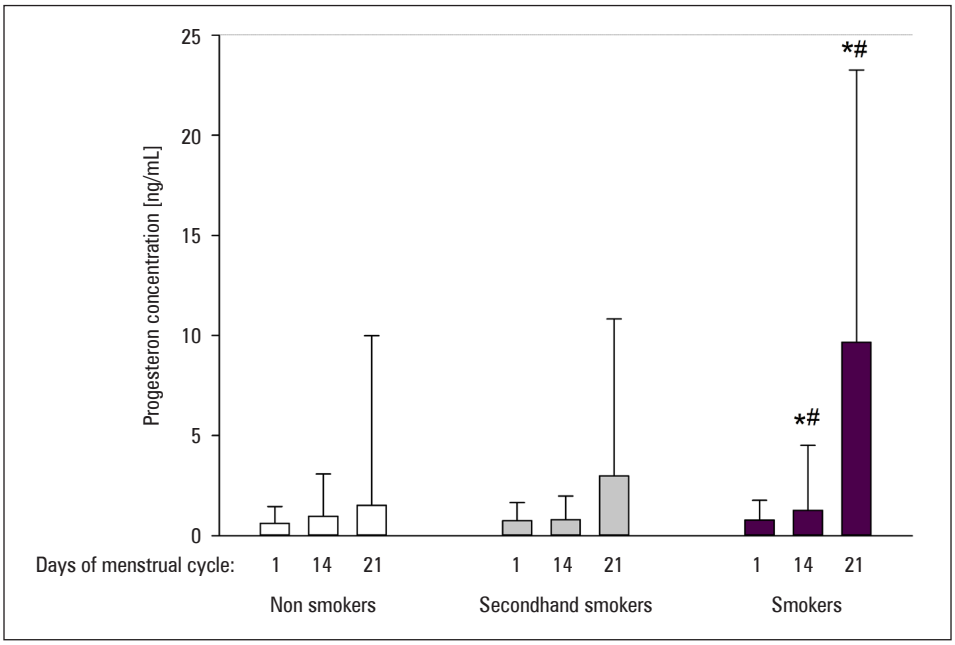

Figure 4. *Mean serum concentrations of progesterone (P) in the groups of non-smoking, exposed to second-hand smoke, and smoking women in the 1st, 14th, and 21st day of the menstrual cycle. *Statistically significant difference between smokers and second-hand smokers (14th day: p < 0.001; 21st day: p = 0.004); #Statistically significant difference between smokers and non-smokers (14th day: p = 0.02; 21st day: p = 0.01)*

(Fig. 3). The level of P (0.75 ng/mL) was seen on the 1st day and on the 14th day (0.80 ng/mL), and the highest values (2.99 ng/mL) were noted on the 21st day of the menstrual cycle (Fig. 4).

Smokers

FSH levels on the 1st and 14th days of the menstrual cycle were 6.02 mIU/mL and 5.02 mIU/mL, respectively, followed by a decrease on the 21st day (2.98 mIU/mL)

Table 2. *Comparison of hormone levels in experimental groups on the 1st day of the cycle*

Hormone	Significance of the Kruskal-Wallis test (p)	Significance of differences (p)		
		0 *vs.* 1	1 *vs.* 2	0 *vs.* 2
FSH	0.03	NS	0.02	NS
LH	0.03	NS	0.03	NS
E2	NS	NS	NS	NS
P	NS	NS	NS	NS

The differences between group 1 and 2 in serum level of FSH ($p = 0.02$) and in LH ($p = 0.03$) was statistically significant. 0 — non-smokers; 1 — second-hand smokers; 2 — smokers; FSH — follicle-stimulating hormone; LH — luteinizing hormone; E2 — 17β-oestradiol; P — progesterone

Table 3. *Comparison of hormone levels in experimental groups on the 14th day of the cycle*

Hormone	Significance of the Kruskal-Wallis test (p)	Significance of differences (p)		
		0 *vs.* 1	1 *vs.* 2	0 *vs.* 2
FSH	0.15 NS	NS	NS	NS
LH	0.16 NS	NS	NS	NS
E2	0.02	NS	NS	0.02
P	< 0.001	NS	< 0.001	0.02

There was a significant relationship between non-smokers (0) and smokers (2) in the serum E2 levels ($p = 0.02$) and in P levels ($p = 0.2$), and in second-hand smokers (1) and smokers (2) in the level of P ($p < 0.001$). 0 — non-smokers; 1 — second-hand smokers; 2 — smokers; FSH — follicle-stimulating hormone; LH — luteinizing hormone; E2 — 17β-oestradiol; P — progesterone

(Fig. 1). The level of LH in the serum of tobacco smoking women and those exposed to the second-hand smoke or non-smoking women is shown in Figure 2.

The level of LH on the 1st day of the menstrual cycle was 4.89 mIU/mL, peak values were reached on the 14th day (10.40 mIU/mL), and then were followed by a pronounced decrease on the 21st day (4.74 mIU/mL). The concentrations of E2 amounted to 42.49 pg/mL, 174.50 pg/mL, and 141.15 pg/mL, respectively (Fig. 3). In the group of smoking women, the levels of P were 0.78, 1.27, and 9.66 ng/mL on the 1st, 14th, and 21st day of the cycle, respectively (Fig. 4).

Significant differences were found ($p = 0.02$) in serum levels of FSH between tobacco-smoking women compared to women exposed to second-hand smoke on the 1st day of the menstrual cycle (Tab. 2) (Fig. 1).

Significant differences were found ($p = 0.03$) in serum levels of LH between tobacco-smoking women and those exposed to second-hand smoke on the 1st day of the menstrual cycle. The level of LH was lower in the group exposed to second-hand smoke than in the smoking group (4.27 mIU/mL, 7.27 mIU/mL, and 5.65 mIU/mL as compared to 4.89, 10.40, and 4.74 mIU/mL, respectively) (Tab. 2) (Fig. 2). Significant differences were also found ($p = 0.02$) in serum levels of E2 between tobacco-smoking women and non-smoking women on the 14th day of the menstrual cycle (Tab. 3) (Fig. 3).

On the 14th day of the menstrual cycle, statistically significant lower levels of P (p 0.001) were detected in the serum of women exposed to second-hand smoke (0.8 ng/mL) and non-smoking women (0.96 ng/ mL) ($p = 0.02$) compared with smoking women (1.27 ng/mL) (Tab. 3). On the 21st day, lower levels of P were found in the group exposed to second-hand smoke (2.99 ng/mL) ($p = 0.004$) and non-smoking women (1.52 ng/mL) ($p = 0.01$) compared to the smoking group (9.66 ng/mL). The differences were statistically significant (Tab. 4) (Fig. 4).

Discussion

Our data showed that the results of the questionnaire were generally related to the cotinine levels in the women's serum on the 1st, 14th, and 21st days of the menstrual cycle, although it appears that some women provided an incorrect history; this was clarified using biomarkers. Therefore, patients previously considered to be non-smokers (primary according to FTND) were re-classified as smokers. This confirms the need to verify the questionnaire survey results using biomarker levels.

The levels of FSH in urine samples increased in a manner appropriate for the follicular phase up to a peak in the periovulatory period. In the case of tobacco smokers, FSH levels changed in a rhythmic manner, but the detected hormone concentrations were higher than those in non-smoking women [32]. An abbreviated follicular phase for tobacco-smoking women, as an effect of higher FSH concentration, was

Table 4. *Comparison of hormone levels in experimental groups on the 21st day of the cycle*

Hormone	Significance of the Kruskal-Wallis test (p)	Significance of differences (p)		
		0 *vs.* 1	1 *vs.* 2	0 *vs.* 2
FSH	0.06 NS	NS	NS	NS
LH	0.15 NS	NS	NS	NS
E2	0.19 NS	NS	NS	NS
Progesterone	0.001	NS	0.004	0.01

The serum P levels (Kruskal-Wallis) were statistically significant between smokers (2) and non-smokers (0) ($p = 0.01$) and smokers (2) and second-hand smokers (1) ($p = 0.004$). 0 — non-smokers; 1 — second-hand smokers; 2 — smokers; FSH — follicle-stimulating hormone; LH — luteinizing hormone; E2 — 17β-oestradiol; P — progesterone

noted by Cramer and De Souza [33, 34]. An increase in FSH induced a more rapid recruitment and development of follicles, which accelerated ovulation [35]. However, an abbreviation of the follicular phase may lead to an abnormal development of follicles, resulting in an abnormal function of the corpus luteum and in inhibition of progesterone secretion [36, 37]. It follows that tobacco smoke directly shortens the menstrual cycle, due to a disturbed secretion of FSH and P, and it promotes decreased fertility in healthy women, which was confirmed by our research and was "enhanced" by serum levels of cotinine. The lowered production of P by the corpus luteum, particularly sensitive to the action of tobacco smoke alkaloids, induced an increase in FSH concentration in the follicular phase due to the feedback loop with the pituitary. Mitra conducted studies on a group of 126 non-smoking and 178 smoking women and demonstrated significantly higher levels of FSH and LH in the group of smokers [38]. Indeed, there is convincing evidence that nicotine has a clear effect on the endocrine system, leading to an increase in FSH, E2, and P [39, 40]. However, we did not find significant differences in serum FSH on the 14th and 21st day of the menstrual cycle between non-smoking, exposed to second-hand smoke, and smoking women, but the differences between second-hand smokers and smokers were statistically significant on the 1st day of the cycle.

The investigations of Duskova et al. [41] demonstrated higher levels of LH as well as lower concentrations of oestradiol in the luteal phase. We observed significant alterations of LH levels in the follicular phase of the menstrual cycle of smoking patients and those exposed to second-and smoke. In the serum of smokers, the LH levels were around 40% higher than in the group exposed to second-hand smoke. The increase in LH on the 1st day of the cycle may suggest increased ovarian resistance. Moreover, it may additionally indicate so-called "poor respondents".

The most important variable influencing the rate of xenobiotics elimination, including tobacco smoke, was the effect on cytochrome P450 enzymes [42, 43]. Tobacco smoke-dependent changes in cytochrome P450 1A1, 1A2, and 2E1 expressions were found in foetuses, newborns, pregnant rats, and human placenta. They are responsible for an increased turnover of oestrogens [44, 45]. Spink et al. [46] showed that tobacco smoking resulted in a deficit of oestrogens, which may reflect an increased glycosylation in positions C-2, C-4, C-15a, and C-6a of E2, through activation of CYP1A [47]. In vitro studies demonstrated a decreased production and turnover of E2 in Graafian follicles under the effect of tobacco smoke alkaloids [48]. Nicotine and its derivatives lead to inactive aromatase, responsible for conversion of androgens to oestrogens [47, 49, 50]. Soldin's et al. [51] results pointed to lower levels of 17-β-oestradiol (E2), oestrone (EN), oestriol (EL) in the serum of smoking women and in those exposed to second-hand smoke, as compared to non-smokers. In our study, statistically significant differences were not observed, but the increase in E2 levels was accompanied by significantly lower serum cotinine concentrations in tobacco smokers.

Our research confirms that smoking women have elevated levels of FSH, which are likely to cause an abnormal ovarian recruitment process and changes in the functioning of the hypothalamic–pituitary–ovarian axis [52, 53]. Research conducted by us is the only one in the world to measure the pituitary (LH and FSH) and P and E2 in three phases and to use the marker of cotinine; also, past studies were conducted on animal models. Moreover, based on cotinine, we have unequivocally proven that smoking leads to ovarian dysfunction and thus to disorders in the synthesis and secretion of hormones such as P and E2. Our research has unequivocally demonstrated abnormal LH activity, which resulted in reduced progesterone levels in smoking women.

However, the significant differences were demonstrated between P levels in the serum of smoking women and those exposed to second-hand smoke on the 14th day of the menstrual cycle. The level of P found in smoking women during the ovulation phase

was almost twofold higher than in individuals exposed to second-hand smoke. This may be explained by the shortening of the follicular phase and earlier ovulation in some women in this group. The presence of cotinine in granulosa follicular fluid was experimentally confirmed in studies conducted by Miceli et al. [54], similarly to the correlation between levels of the nicotine metabolite in follicular fluid in serum of smoking women. The inhibition of P secretion by nicotine and its metabolites resulted from disturbances in prostaglandin metabolism in the corpus luteum, and from inhibition of its secretion. Prostaglandin F_2a (PG F_2a) induces structural and functional regression of the corpus luteum while prostaglandin E_2 (PG E_2) manifests luteotropic effects and induces expression of genes coding for enzymes involved in steroidogenesis. The disturbances lead to decreased P levels both in the luteal phase, in early pregnancy, and in ovarian hyperstimulation syndrome (OHS) [54–56].

We have shown that the P levels in smokers and non-smokers or passive smokers on the 14th day of the cycle have no significant clinical implications, although a statistically significant relationship was found ($p = 0.02$ and $p < 0.001$, respectively). However, a very significant correlation in P concentration on the 21st day of cycle was found, which may indicate an impaired secretory function of the corpus luteum. Our research is important because it clearly shows a negative effect of nicotine on P levels in the luteal phase, and a statistically significant difference between smokers and non-smokers (0) ($p = 0.01$) and between smokers (2) and second-hand smokers (1) ($p = 0.004$), which may indicate a lower quality of egg cell and dysfunction of the corpus luteum.

The differences that were observed during our study compared to the observations of other investigators may be due to other factors related to the menstrual cycle, which we are not able to eliminate, such as environmental factors (climate change, time zones, diet, and stress), and genetic predisposition [57]. In the future, research should take into account the narrower age divisions in the study group of women in reproductive age [58].

Tobacco smoking remains a significant medical problem, especially because it relates to fertility (e.g. premature ovarian failure) and in particular for females who smoke tobacco. Our study is even more interesting because we are the first to perform hormonal analysis (LH, FSH, P, E2) in women of childbearing age in every phase of the menstrual cycle, for smokers, females passively exposed to tobacco, and non-smokers. In addition to the Fagerström Test for Nicotine Dependence (Fagerström), we analysed the concentration of cotinine in the serum (a biomarker of exposure to tobacco smoke) [59–61].

Conclusions

In smoking patients, the serum level of LH are significantly higher in the first days of the menstrual cycle, manifesting the potential for disturbing ovulation, and resulting in insufficiency of the luteal phase. The higher levels of P in midluteal phase have been assumed to be the reason for the longer menstrual cycle and disturbances in cycle regularity declared by the patients. The increase in oestradiol E2 level is accompanied by significantly lowered serum cotinine concentrations in tobacco smokers (negative R_s). This is probably the result of slowed nicotine metabolism or from the accelerated biotransformation of cotinine.

Cigarette smoking is an important factor in disrupting reproduction; hence, it is necessary to provide medical education to women of reproductive age about the potential dangers of tobacco smoke to fertility.

Conflicts of interests

The authors declare that there are no conflicts of interest.

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