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The Effect of Varenicline on The Ovarian Follicle and Hormones, and Endometrial Thickness In An Experimental Model

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ABSTRACT

It was aimed to evaluate the histological, biochemical, and hormonal changes in the reproductive system of varenicline exposed female rats in an experimental model. Forty female rats were randomized into 4 groups. (1) Control group (n = 10); (2) Smoking group (smoking exposure 30 minutes twice a day) (n = 8); (3) Varenicline group (1 ml/kg); (4) Smoking+Varenicline (varenicline treatment (1 ml/kg) after smoking exposure (30 minutes twice a day). After the experiment, ovarian follicle count and endometrial thickness were examined; biochemical hormones -include Anti-mullerian hormone- and free oxygen radical levels were calculated. AMH levels were significantly lower in group 4 than in group 1 (p = 0.049).

Group 2 and group 3 were significantly lower than group 1 (p = 0.038, p = 0.045, respectively). There was no statistically significant difference in endometrial thickness and free oxygen radical levels between the groups (p>0.05).

We found that varenicline is related to reduced AMH and PRMF levels. The difference in endometrial thickness between the groups was not detected. These findings show that varenicline may harm ovarian functions.

Keywords: Smoking, varenicline, oxidative stress, AMH, primordial follicle

Introduction

Smoking addiction is one of the most important preventable social problems. Approximately 1 billion people smoke worldwide, 800 million of whom are in developing countries (1,2).

The effects of cigarettes on female reproductive life are revealed by many studies (3-7). It has been reported that smoking exposure is associated with premature loss of ovarian function and decreased fertility (3). Experimental studies have shown that there is a relationship between nicotine exposure and follicular loss (4). Cigarette smoking has been shown to cause infertility in women by reducing folliculogenesis, steroidogenesis, and tubal activity (5-7). However previous studies reported smoking may be related to a dose-dependent reduction of endometrial proliferation, the consequent effect is unclear (8, 9).

Cigarette smoke contains further than 4000 biochemical active agents which include toxic and carcinogenic compounds. Studies suggest that some of these compounds may disrupt ovarian function through oxidative damage by producing reactive oxygen species (ROS) (10, 11). Increased oxidative status causes cell damage with affect DNA, protein, and lipids. Prooxidant/oxidant balance is important for improving ovarian follicles and the result of impaired balance is slowing oocyte maturation (12).

Varenicline is a synthetic partial agonist of α4β2 and α3β4 nicotinic cholinergic receptor that is commonly targeted in the treatment of cigarette addiction in recent years which is proven superior to other pharmacological drugs (13). As a partial agonist, varenicline binds to nicotinic cholinergic receptors and induces receptor activation, leading to dopamine release (agonistic effect) and

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inhibiting cholinergic receptor binding of exogenous nicotine (antagonistic effect). Thus, abstinence symptoms and smoking wishes are reduced (14).

In the current literature, there is no information about varenicline therapy and its effects on the ovary and endometrial physiology. This study aims to evaluate the histological, biochemical, and hormonal changes in the reproductive system of varenicline exposed female rats in an experimental model.

Materials and Methods

The study was carried out after the approval of the local ethics committee (Date of 15.04.2015 and 15/39 decision no.) in Kırıkkale University Scientific and Technological Research Laboratories. All animal experiments were executed following the principles and guidelines for the use of laboratory animals (15). For this study, a total of 40 Wistar-Albino female rats weighing 300-350 g were obtained from the Animal Laboratory. Rats were housed in plastic cages until the study was performed; and were given standard rodent diet and water at an appropriate suitable temperature (24-25 ° C), damp range medium (55-60%), and controlled light (12:12 hours light: dark).

Groups: Rats were divided into randomly 4 groups:

Group 1: Negative control group (no intervention was applied except standard care of animals, n = 10),

Group 2: Smoking Group (n = 10) (standard care in addition to smoking exposure,30 minutes twice per dayfor1 month),

Group 3: Varenicline Group (n = 10) (standard care in addition to only varenicline treatment, 1ml/kg for 2 weeks),

Group 4: Smoking + Varenicline Group (n = 10)(standard care in addition to varenicline treatment (1 ml/kg for2 weeks) after cigarette exposure (30 minutes twice per day for1 month) (Table 1).

Before the experiment, all of rats were monitored daily by vaginal smear to hormonally synchronize estrous cyclicity. Thus, differences in ovarian morphology and steroidogenesis were excluded. Animals that had a regular estrous cycle were utilized for the experiment.

Experimental Procedure: For the 2 groups of rats to be exposed to cigarette smoke, 160x130x60 cm transparent plastic, 2 lids on the front, and 2 cages with 20 □ 20 cm opening and closing windows were made. A mechanism was installed using the aquarium engine and the rats were exposed to cigarette smoke for 30 minutes in the morning and evening. Camel Black™ (10 mg, 0.8 mg nicotine) was used as a cigarette. Varenicline (Champix, Pfizer Manufacturing Deutschland GmbH Heinrich-Mack-Str. 35 89257Illertissen /Germany) 1 mg 28 tablets, Pfizer) was diluted with 0.9% saline and subcutaneously administered at 1 ml/kg dose at 09:00 a.m and 18:00. Cigarette exposure and varenicline doses were carried out as described previously by researchers (16, 17).

At the end of the 6-week study, surgical procedures were performed in the laboratory and sterile conditions. Under general anesthesia □ ketamine hydrochloride (Ketalar flacon; Pfizer, Istanbul, Turkey) 50 mg/kg intraperitoneal, a longitudinal incision was made in the abdominal region and entered into the abdomen. Blood was taken from vena cava inferior for biochemical studies. Hysterectomy-include two horns of uterus-and right oophorectomy were performed according to the procedure and were fixed in 10% formalin. After the surgical procedure, rats were sacrificed by decapitation.

Histopathological examinations: Histological examination was performed at the pathology department of University Hospital. Ovarian tissue samples and uterus were fixed with 10% formalin solution and whole layer cross-sections were obtained. Then all tissues were embedded in paraffin blocks. Tissues were sectioned in 5 µm pieces and were stained using the routine hematoxylin-eosin staining. The morphological observation was carried out blindly and a light microscope (Lecia DM 2500, Germany) was used for examination of the specimen.

Follicles were defined as the classification of Erickson (18): 1. Primordial follicle; if an oocyte is surrounded by a single layer of flat epithelial cells, 2. Primary follicle; if an oocyte is surrounded by a single layer of cubic to high-prismatic cells, 3. Secondary follicle; if the oocyte is increased and follicle consists of at least two cell layers. Zona pellucida and theca follicle should be seen, 4. Tertiary follicle; if the follicle had an antrum, stratum granulosum layer, and cumulus oophorus. Pederson and Peters' modified method was used to determine ovarian follicle count (19). The endometrial thickness of the rat was measured in micrometers.

Biochemical examinations: Blood samples in non-heparin tubes were centrifuged in cold at

1500 □ g for 15 minutes. Supernatant serums were placed into Eppendorf Tubes and kept at - 80 □ C until assay.

Plasma AMH level was measured by ELISA method (Bioassay, China) according to manufacturer's instructions. FSH, LH, E2 levels were detected by the auto-analyzer (BeckmanCoultarDXI-600). Total-SH and MDA were measured by spectrophotometric methods and free oxygen radical levels of the sample were determined.

Statistical Analysis: The statistical analysis was performed using the Statistical Package for the Social Sciences version 21 (SPSS Inc., Chicago, IL). Data were presented as mean ± SD. For each continuous variable, normality was checked by the Shapiro Wilks test. Continuous variables were compared by Kruskal-Wallis H test for non-parametric data with a post hoc analysis using a Mann-Whitney U test. For parametric data, One-way ANOVA followed by Bonferroni post hoc test was used. $p \leq 0.05$ was considered statistically significant.

Results

2 rats died during the experiment in group 2 (n = 8).

Levels of AMH, FSH, LH, and E2 were shown in Table 2 and follicular counting was shown in Table 3.

AMH, FSH, LH, E2 and PRMF levels were different between the groups (p = 0.05, 0.02, < 0.001, 0.002 and 0.018, respectively).

The post hoc test result, AMH levels were significantly lower in group 4 than in group 1 (p = 0.049). (Figure1)

PRMF levels were the highest in group 1. Group 2 and group 3 were significantly lower than the group 1 (p = 0.038, p = 0.045, respectively). The numbers of other growing follicles weren't different in groups (p > 0.05). (Figure1-2)

There was no statistically significant difference in endometrial thickness between the groups (p > 0.05).

FSH and E2 levels were found highest in group 1. Group 4 was significantly lower than group 1 for FSH and E2 levels (p = 0.08, p = < 0.001, respectively).

E2 levels in group 2 and group 3 were significantly higher than group 4 (p = 0.033 and 0.007, respectively).

LH levels in group 4 were significantly higher than group 2 and group 3 (p = 0.036, 0.004 respectively).

There was no statistically significant difference between the MDA and Total-SH levels of the oxidative markers (p > 0.05).

Discussion

To our knowledge, our study is the first study for the effects of varenicline, which is used for smoke addiction treatment, on woman reproductive physiology. We suggest that varenicline is related to reduced AMH and PRMF levels. The effect of varenicline on the endometrium is unclear. We did not detect any difference in endometrial thickness between the groups. These findings show that varenicline may harm ovarian functions.

Smoking is a health problem that affecting reproductive functions and fertility (20-23). It is known that smoking addiction is related to decreased fecundability rate, slowing down the ovarian follicle growth, and premature menopause in women in their reproductive age (24, 25). It has also been shown that cigarette smoking causes a decrease in the number of oocytes retrieved and fertilization-implantation rates (26, 27). Although cigarette negatively affects whole reproductive physiology according to the current knowledge, the exact mechanism of smoking-associated adverse effects is not clearly understood yet.

Studies have shown that the best biochemical marker for showing ovarian reserve is AMH, a glycopeptide released from the direct ovary (28). When quantitative responses to gonadotropin stimulation were analyzed in histologically evaluated primordial follicle counts and IVF treatment, AMH and antral follicle count evaluated by sonography were reported to give the most accurate estimation on ovarian reserve (29, 30). Smoking has been shown to reduce AMH levels and primordial follicle counts by decreasing ovarian reserve (31, 32).

Cigarette exposure is considered to disrupt reproductive function through primordial function depletion and caused ovo-toxicity. The result of primordial follicle depletion is caused to early menopause and associated with loss of reproductive potential (25, 33). Smoking decreases all stages of folliculogenesis, but the reduction in the primordial follicle may be particularly important as it may indicate a decrease in ovarian reserve. In our study, the highest AMH and primordial follicle levels were determined in the

Table 1: The 6-Weeks Study Plan of The Groups

Groups/ Weeks	1	2	3	4	5	6
Control	-	-	-	-	-	-
Smoking	-	-	S	S	S	S
Varenicline	-	-	-	-	V	V
Smoking+ Varenicline	S	S	S	S	V	V

S: Smoking exposure V: Varenicline treatment

Table 2: Comparison of Follicular Counting and Endometrial Thickness Into The Groups

	Group 1 (Sham) n = 10 (Mean ± SD)	Group 2 (Smoking) n = 8 (Mean ± SD)	Group 3 (Varenicline) n = 10 (Mean ± SD)	Group 4 (Smoking+ Varenicline) n = 10 (Mean ± SD)	p value
Primordial (n)	9.7 ± 2.9 a, b	7.8 ± 5.0	5.4 ± 2.3 a	5.50 ± 2.7 b	0.018*
Primer (n)	6.4 ± 2.2	4.9 ± 3.4	4.1 ± 2.8	4.6 ± 3.0	NS
Seconder (n)	7.7 ± 4.0	7.6 ± 5.8	7.3 ± 4.6	12.9 ± 5.9	NS
Tertiary (n)	6.2 ± 3.2	5.4 ± 4.2	5.0 ± 3.8	6.5 ± 4.6	NS
Corpus Luteum (n)	6.2 ± 4.5	4.4 ± 4.7	5.2 ± 2.9	9.6 ± 5.4	NS
Endometrial thickness (μm)	259.0 ± 79.7	252.6 ± 83.6	268.1 ± 48.3	295.9 ± 80.4	NS

Each different lower-case letter defines significant difference among columns [a] p = 0.038,[b] p = 0.045, NS = Non-significant (p > 0.05)

Table 3: Biochemical Levels Into The Groups

	Group 1 (Sham) n = 10 (Mean ± SD)	Group 2 (Smoking) n = 8 (Mean ± SD)	Group 3 (Varenicline) n = 10 (Mean ± SD)	Group 4 (Smoking+ Varenicline) n = 10 (Mean ± SD)	p value
AMH (ng/mL)	1.50 ± 0.46[a]	1.40 ± 0.40	1.27 ± 0.34	1.01 ± 0.36 [a]	0.05*
FSH (IU/mL)	7.50 ± 4.54[b]	5.39 ± 4.03	6.57 ± 3.76 [c]	2.94 ± 2.07[b, c]	0.02*
LH (IU/mL)	0.36 ± 0.32[d]	0.26 ± 0.29 [e, f, g]	0.95 ± 0.10[d, e]	0.65 ± 0.29 [f, g]	< 0.001*
E2 (ng/mL)	89.62 ± 11.34 [d]	79.93 ± 11.90 [h]	80.88 ± 11.55 [ı]	58.70 ± 20.79[d, h, ı]	0.002*
MDA (nmol/mL)	87.90 ± 53.02	80.40 ± 86.85	61.32 ± 43.34	49.23 ± 39.34	NS
Total-SH (μmol/L)	1250.90 ± 897.76	802.38 ± 316.49	1028.20 ± 363.73	623.50 ± 202.88	NS

Each different lower-case letter defines significant difference among columns [a] p = 0.049,[b] p = 0.008, [c]p = 0.013,[d]p < 0,001,[e] p = 0.003, [f]p = 0.036, [g]p = 0.004, [h] p = 0.033, [I]p = 0.007, NS = Non-significant (p > 0.05).

control group. This result also supports the previous studies on cigarette and ovarian reserve.

We also found that the AMH and E2 levels in the smoking-varenicline group were significantly lower than the control group and there was no statistically significant difference between the smoker group and the control groups. In the histopathological examination of the ovaries, only varenicline treatment and smoking-varenicline

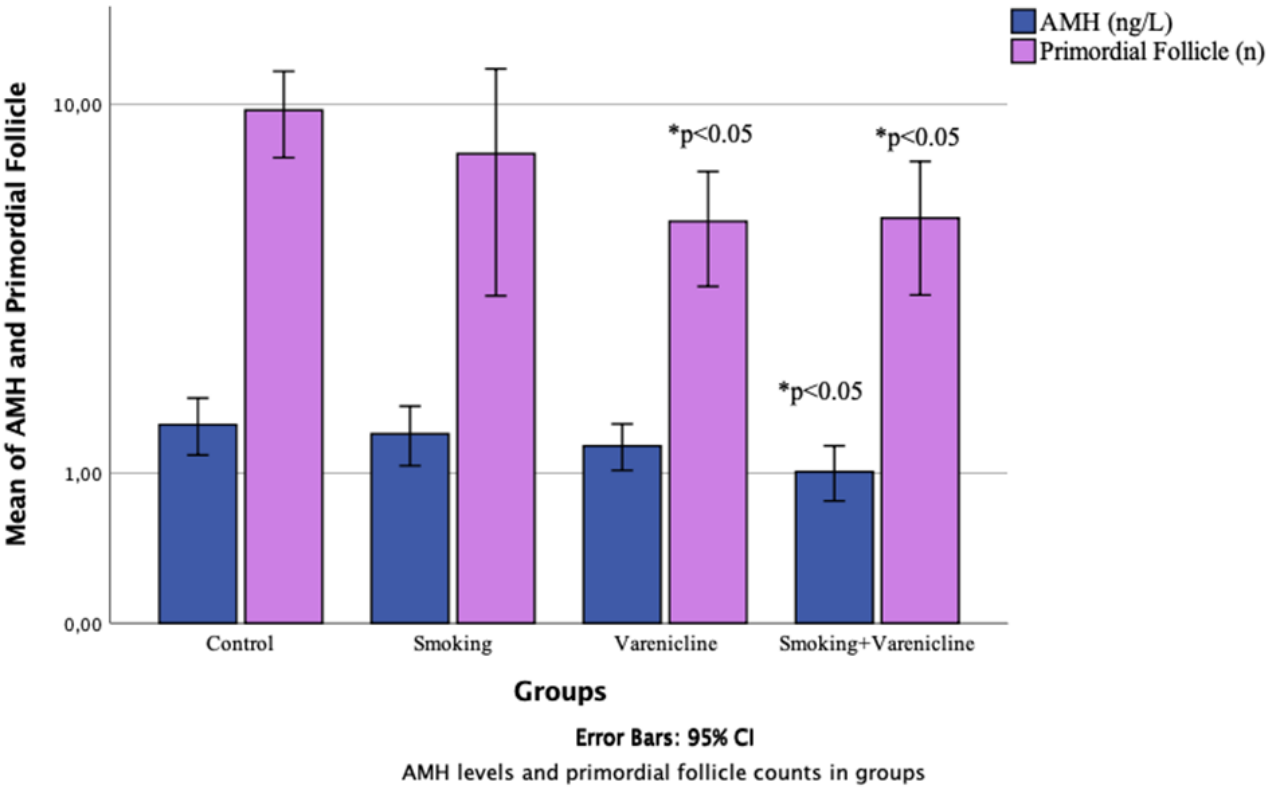

Fig. 1. AMH levels and Primordial Follicle Counts In Groups

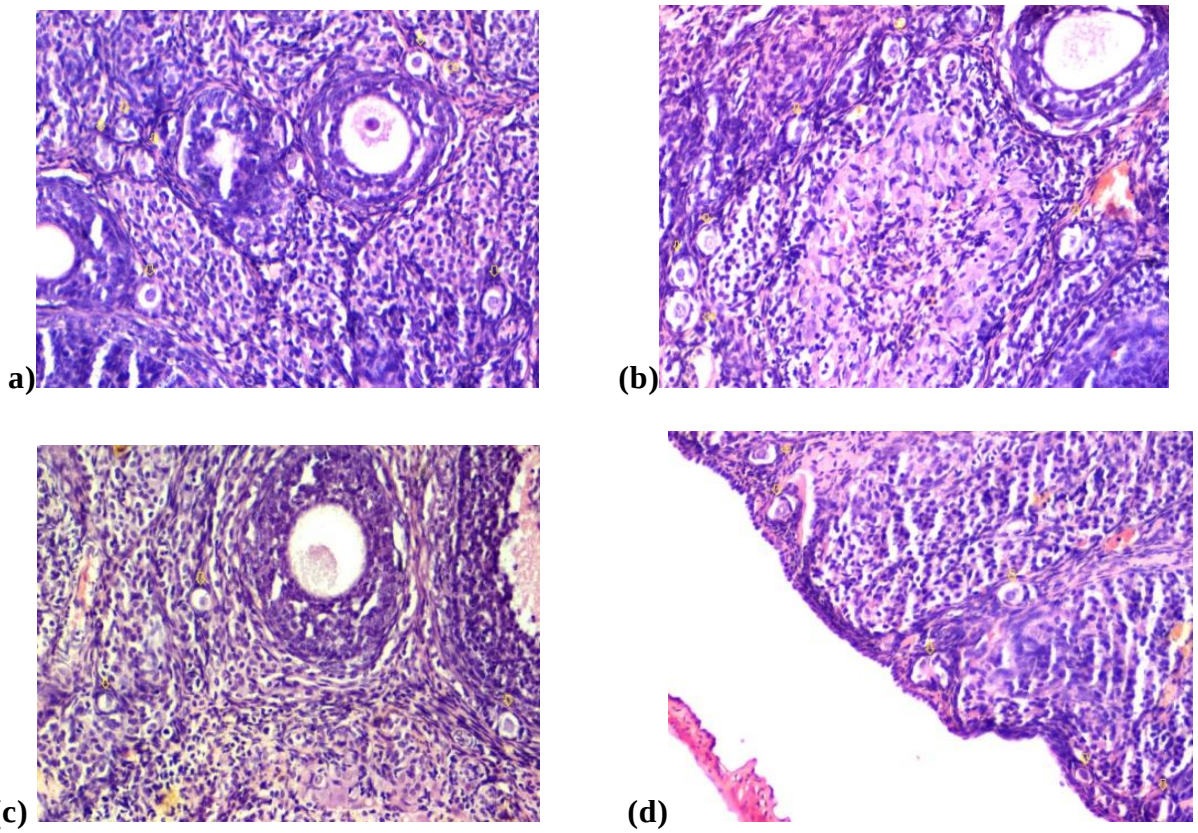

Fig. 2. Primordial follicles in groups. (a) group 1, (b) group 2, (c)group 3, (d)group 4. Yellow arrows show primordial follicles which surrounded by a single layer of flat epithelial cells (hematoxylin eosin [H-E]; X200

group PRMF values were significantly lower than the control group.

In this study, we observed acute effects of varenicline. It was seen that the treatment of varenicline with/without smoking caused decreased ovarian primordial follicle and E2 levels. However, the atrophic effect of this result on endometrial thickness was not statistically reflected. We could not find a statistical difference in oxidative markers, and this result suggests that the ovarian effects of varenicline might not be related to the antioxidant system. Studies investigating the effect of smoking on endogenous hormones have produced conflicting results. Some have found no association between smokers and non-smokers, but others have found a relationship between smoking and FSH, LH, E2, progesterone, and SHBG (34, 35).

FSH and LH levels are increased in smokers but some studies have found that pituitary gland hormones are not affected by cigarette smoking (36-38). Cigarette compounds are suspected to alter hormone function (39). Smoking may affect hormone function in premenopausal women (40). Some studies showed higher FSH and LH levels in smokers while some others observed no significant difference between smokers and non-smokers. In our study, both smoking and smoking-varenicline group FSH values were lower than the control group but the only smoking-varenicline group is statistically significant compared to the control group. Smoking-varenicline group LH values were not statistically significant compared to the control group.

Cigarettes lead to a lot of toxic chemicals and pro-oxidants that can be transformed into reactive oxygen species (ROS). There have been several studies that have shown cigarette smoking has a detrimental effect on tissues by causing oxidative damage (41, 42). Smoking is linked to infertility through the activation of ROS (43, 44). It was observed that Malondialdehyde (MDA) levels increased and Total Sulfhydryl (Total-SD) levels were negatively affected in smoking women. (45, 46). Although the studies, we could not detect a significant statistical difference in MDA and Total-SH levels between the groups.

As a result, this study is the first study that examined the effect of varenicline on female reproductive functions and our findings show that varenicline therapy does not reverse the negative effect of cigarettes on women's health. On the contrary, we think that the use of varenicline disrupts ovarian function by decreasing AMH and PRMF levels.

We found that the ovarian- reserve in the group treated with varenicline treatment after smoking was lower than that of the smoking group only. The detection of FSH and E2 levels in the lowest varenicline + cigarette group supported this assertion and more extensive researches are needed in this regard.

Conflict of Interest: The authors declare no conflict of interest in this paper.

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Author contributions section:

Murat Bulanık: Creating the study protocol and writing the article,

Nevin Sağsöz: Creating the study protocol and writing the article,

ŞükrüBakırcı: Writing the article,

MahmutİlkinYeral: Writing the article,

PınarAtasoy: Examination and interpretation of pathological specimens,

HakanBoyunağa: Examination and interpretation of biochemical parameters.

References

1. Karnath B. Smoking cessation. American Journal of Medicine 2002; 112 (5): 399-405.
2. WHO International Agency for Research on Cancer, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans 2004; 83: 97-102.
3. Hughes EG, Lamont DA, Beecroft ML, Wilson DM, Brennan BG et al. Randomized trial of a 'stage-of-change' oriented smoking cessation intervention in infertile and pregnant women Fertility and Sterility 2000; 74 (3): 498-503.
4. Mulligan Tuttle AM, Stampfli M, Foster WG. Cigarette smoke causes follicle loss in mice ovaries at concentrations representative of human exposure. Human Reproduction 2009; 24 (6):1452-1459.
5. de Mouzon J, Belaisch-Allart J. Consequences on women's fecundity and on assisted reproductive technology. Journal de GynécologieObstétrique et Biologie de la Reproduction 2005; 34 (1): 112–118.
6. Wright KP, Trimarchi JR, Allsworth J, Keefe D. The effect of female tobacco smoking on

IVF outcomes. Human Reproduction 2006; 21 (11): 2930-2934.

7. Vidal JD, VandeVoort CA, Marcus CB, Lazarewicz NR, Conley AJ. In vitro exposure to environmental tobacco Reproductive Toxicology 2006; 22 (4): 731-737.
8. Khorram O, Han G, Magee T. Cigarette smoke inhibits endometrial epithelial cell proliferation through a nitric oxide-mediated pathway. Fertility and Sterility 2010; 93 (1): 257-263.
9. ZhangW, Yang J, Wang J, Xia P, Xu Y et al. Comparative studies on the increase of uterine weight and related mechanisms of cadmium and p-nonylphenol. Toxicology 2007; 241 (1-2): 84-91.
10. Sobinoff AP, Mahony M, Nixon B, Roman SD, McLaughlin EA. Understanding the villain: DMBA-induced preantral ovotoxicity involves selective follicular destruction and primordial follicle activation through PI3K/Akt and mTOR signaling. Toxicological Sciences 2011; 123 (2): 563-575.
11. Paszkowski T, Clarke RN, Hornstein MD. Smoking induces oxidative stress inside the Graafian follicle. Human Reproduction 2002; 17(4): 921-925.
12. Sabatini L, Wilson C, Lower A, Al-Shawaf T, Grudzinskas J.G. Superoxide dismutase activity in human follicular fluid after controlled ovarian hyperstimulation in women undergoing in vitro fertilization. Fertility and Sterility 1999; 72 (6): 1027-1034.
13. Bidwell LC, Karoly HC, Hutchison KE, Bryan AD. ADHD symptoms impact smoking outcomes and withdrawal in response to varenicline treatment for smoking cessation. Drug Alcohol Dependence 2017; 179: 18-24. doi: 10.1016/j.drugalcdep.2017.06.020
14. Jorenby DE, Hays JT, Rigotti NA, Azoulay S, Watsky EJ, Williams KE, Billing CB, Gong J, Reeves KE. Efficacy of varenicline, an a4b2 nicotinic acetylcholine partial receptor agonist, vs placebo or sustained release bupropion for smoking cessation: a randomized controlled trial. JAMA 2006; 296 (1): 56-63.
15. Couto M, Cates C. Laboratory guidelines for animal care. Methods in Molecular Biology 2019; 1920: 407-430.
16. Hardaker EL, Freeman MS, Dale N, Bahra P, Raza F, Banner KH, Poll C. Exposing rodents to a combination of tobacco smoke and lipopolysaccharide results in an exaggerated inflammatory response in the lung. British Journal of Pharmacology 2010; 160 (8): 1985-1996.
17. Le Foll B, Chakraborty-Chatterjee M, Lev-Ran S, Barnes C, Pushparaj A, Gamaleddin I, Yan Y, Khaled M, Goldberg SR. Varenicline decreases nicotine self-administration and cue-induced reinstatement of nicotine-seeking behaviour in rats when a long pretreatment time is used. International Journal of Neuropsychopharmacology 2012;15(9):1265-74.
18. Erickson GF, Shimasaki S. The physiology of folliculogenesis: the role of novel growth factors. Fertilty and Sterility 2001; 76(5): 943-949.
19. Pedersen T, Peters H. Proposal for a classification of oocytes and follicles in the mouse ovary. Journal of Reproduction Fertility 1968; 17 (3): 555-557.
20. Whitcomb BW, Bodach SD, Mumford SL, Perkins NJ, Trevisan M, Wactawski-Wende J, Liu A, Schisterman EF. Ovarian function and cigarette smoking. Paediatric and Perinatal Epidemiology 2010; 24 (5): 433-440.
21. Ye X, Skjaerven R, Basso O, Baird DD, Eggesbo M, CupulUicab LA, Haug K, Longnecker MP. In utero exposure to tobacco smoke and subsequent reduced fertility in females. Human Reproduction 2010; 25(11): 2901-2906.
22. Dechanet C, Anahory T, Mathieu Daude JC, Quantin X, Reyftmann L, Hamamah S, Hedon B, Dechaud H. Effects of cigarette smoking on reproduction. Human Reproduction Update 2011; 17 (1): 76-95.
23. Sadeu JC, Hughes CL, Agarwal S, Foster WG. Alcohol, drugs, caffeine, tobacco, and environmental contaminant exposure: reproductive health consequences and clinical implications. Critical Reviews in Toxicology. 2010; 40(7): 633-652.
24. Hull MG, North K, Taylor H, Farrow A, Ford WC. Delayed conception and active and passive smoking. The Avon Longitudinal Study of Pregnancy and Childhood Study Team. Fertility and Sterility 2000; 74 (4): 725–733.
25. Parente RC, Faerstein E, Celeste RK, Werneck GL. The relationship between smoking and age at the menopause: a systematic review. Maturitas 2008; 61 (4): 287-98. .
26. Fuentes A, Munoz A, Barnhart K, Arguello B, Diaz M, Pommer R. Recent cigarette smoking and assisted reproductive technologies outcome. Fertility and Sterility 2010; 93 (1): 89-95.
27. Soares SR, Simon C, Remohi J, Pellicer A. Cigarette smoking affects uterine receptiveness. Human Reproduction 2007; 22 (2): 543-547.
28. La Marca A, Sighinolfi G, Radi D, Argento C, Baraldi E, Artenisio AC, Stabile G, Volpe A. Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive

technology (ART). Human Reproduction Update 2010; 16 (2): 113-130.

29. Hansen KR, Hodnett GM, Knowlton N, Craig LB. Correlation of ovarian reserve tests with histologically determined primordial follicle number. Fertility and Sterility 2011; 95 (1): 170-175.
30. Broer SL, van Disseldorp J, Broeze KA, Dolleman M, Opmeer BC, Bossuyt P, Eijkemans MJ, MolBW, Broekmans FJ;IMPORT study group. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: an individual patient data approach. Human Reproduction Update 2013; 19 (1): 26-36.
31. [Freour T, Masson D, Mirallie S, Jean M, Bach K, Dejoie T, Barriere P. Active smoking compromises IVF outcome and affects ovarian reserve. Reproductive Biomedicine Online 2008; 16 (1): 961102.
32. Neal MS, Zhu J, Holloway AC, Foster WG. Follicle growth is inhibited by benzo-[a]-pyrene, at concentrations representative of human exposure, in an isolated rat follicle culture assay. Human Reproduction 2007; 22 (4): 961-967.
33. Sun L, Tan L, Yang F, Luo Y, Li X, Deng HW, Dvornyk V. Meta-analysis suggests that smoking is associated with an increased risk of early natural menopause. Menopause 2011; 19 (2): 126-132.
34. Tanko LB, Christiansen C. An update on the antiestrogenic effect of smoking: a literature review with implications for researchers and practitioners. Menopause 2004; 11 (1): 104-109.
35. Baron JA, La Vecchia C, Levi F. The antiestrogenic effect of cigarette smoking in women. American Journal of Obstetrics and Gynecology 1990; 162 (2): 502-514.
36. Key TJ, Pike MC, Baron JA, Moore JW, Wang DY, Thomas BS, Bulbrook RD. Cigarette smoking and steroid hormones in women. Journal of Steroid Biochemistry and Molecular Biology 1991; 39 (4A): 529-534.
37. Rannevik G, Jeppsson S, Johnell O, Bjerre B, Laurell-Borulf Y, Svanberg L. A longitudinal study of the perimenopausal transition: altered profiles of steroid and pituitary hormones, SHBG and bone mineral density. Maturitas 2008; 61 (1): 67-77.
38. Sansone A, Di Dato C, de Angelis C, Menafra D, Pozza C, Pivonello R, Isidori A, Gianfrilli D. Smoke, alcohol and drug addiction and male fertility. Reproductive Biology and Endocrinology 2018; 16 (1): 3.
39. Kapoor D, Jones TH. Smoking and hormones in health and endocrine disorders. European Journal of Endocrinology 2005; 152 (4): 491-499.
40. Windham GC, Mitchell P, Anderson M, Lasley BL. Cigarette smoking and effects on hormone function in premenopausal women. Environmental Health Perspectives 2005; 113(10): 1285-1290.
41. Harvilchuck JA, Pu X, Klaunig JE, Carlson GP. Indicators of oxidative stress and apoptosis in mouse whole lung and Clara cells following exposure to styrene and its metabolites. Toxicology. 2009; 264 (3): 171-178.
42. Zhang SP, Wu YW, Wu ZZ, Liu HY, Nie JH, Tong J. Upregulation of rage and S100A6 in rats exposed to cigarette smoke. Environmental Toxicology and Pharmacology. 2009; 28 (2): 259-264.
43. Appt SE, Kaplan JR, Clarkson TB, Cline JM, Christian PJ, Hoyer PB. Destruction of primordial ovarian follicles in adult cynomolgus macaques after exposure to 4-vinylcyclohexene diepoxide: a nonhuman primate model of the menopausal transition. Fertility and Sterility 2006; 86(4 Suppl): 1210-1216.
44. Sobinoff AP, Beckett EL, Jarnicki AG, Sutherland JM, McCluskey A, Hansbro PM, McLaughlin EA. Scrambled and fried: cigarette smoke exposure causes antral follicle destruction and oocyte dysfunction through oxidative stress. Toxicology and Applied Pharmacology 2013; 271 (2): 156-167.
45. Bamonti F, Novembrino C, Ippolito S, Soresi E, Ciani A, Lonati S, Scurati-Manzoni E, Cighetti G. Increased free malondialdehyde concentrations in smokers normalise with a mixed fruit and vegetable juice concentrate: a pilot study. Clinical Chemistry and Laboratory Medicine. 2006; 44(4): 391-395.
46. Solak I, Cetinkaya CD, Gederet YT, Kozanhan B, Erel O, Eryilmaz MA. Effects of smoking on thiol/disulfide homeostasis. European Review for Medical and Pharmacological Sciences. 2018; 22(8): 2477-2482.