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Impact of different typologies of smoking on ovarian reserve and oocyte quality in women performing ICSI cycles: an observational prospective study

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Abstract. – OBJECTIVE: Within the last few years smoking activities, as well as infertility, have increased in Italy, and so has the consumption of alternative cigarette devices among women of childbearing age. The aim of this observational study was to evaluate the impact of the consumption of cigarettes and alternatives devices, such as electronic cigarettes and heat-not-burn (HnB) products, on infertile women performing in vitro fertilization (IVF), in specific on the quality of oocytes retrieved in women performing intracytoplasmic sperm injection (ICSI) cycles.

PATIENTS AND METHODS: Prospective observational longitudinal study involving 410 women referring to the Reproductive Physiopathology and Andrology Unit, Sandro Pertini Hospital, Rome, from 2019-2022. All the women enrolled filled out an elaborate questionnaire investigating smoking consumption, before the beginning of ovarian stimulation by antagonist protocol, ovarian pick-up, and subsequent ICSI technique. The outcomes of the study were the evaluation of clinical and ICSI features between the groups of smokers and non-smokers: the number of retrieved oocytes, immature oocytes, and fertilization rate were confronted between the two groups and between cigarette smokers *vs*. e-cigarette and heat-not-burn (HnB) products smokers.

RESULTS: Clinical parameters were comparable between the group of smokers compared to one of the non-smokers, except for anti-Müllerian hormone (AMH), which was statistically lower in smokers ($p<0.05$). Regarding IVF hormonal stimulations it appears that the total dose of gonadotropin was statistically lower in the non-smoker's group, compared to smokers (1850±860 UI *vs*. 1,730±780 $p<0.05$). Regarding ICSI techniques interestingly the number of oocytes retrieved was lower in the smokers' group compared to non-smokers (5.21±0.9 *vs*. 6.55±3.5, $p<0.001$), and the number of empty zona pellucida oocytes was statistically higher in the smokers' group (0.51±0.1 *vs*. 0.2±0.1, $p<0.05$). On the other hand, the fertilization rate (FR) was statistically higher in non-smokers compared to the smokers' group (72.16±3.05 *vs*. 68.12±2.21, $p=0.03$). Out of the 203 smokers, overall, any statistically significant difference, regarding ICSI results, has been found between the group of cigarette smokers, compared to the group of e-cigarettes plus HnB products smokers.

CONCLUSIONS: Smoking negatively impacts human fertility, leading to a reduction of ovarian reserve and ovarian quality, which can negatively impact results in women performing ICSI cycles. Despite the limitation of the study, our results underline that consumption of cigarette alternative devices seems to have a similar negative impact on the quantity and quality of oocytes retrieved in ICSI cycles. Clinicians should emphasize the reduction of exposure to harmful substances derived from the combustion of tobacco smoking, as well as alternative devices, in women of childbearing age.

Key Words:
Cigarette smoking, Electronic cigarettes, Heat-not-burn products, ICSI, Infertility, Ovotoxicity, Ovarian reserve.

Introduction

Infertility has been increasing worldwide and so has in Italy, where at least 1/7 heterosexual couples experience reproductive problems, with more than 77,000 of them performing *in vitro* fer-

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tilization (IVF) techniques and 14,000 children born from IVF techniques every year[1,2]. Among unhealthy lifestyles, cigarette tobacco smoking represents the most prominent hazard to public health in the world and in Italy, where it is the major risk factor causing early death and disability for all ages[3]. Indeed, it is estimated that in Italy over 93,000 deaths are attributable to tobacco smoking, specifically 20.6% of the total among men and 7.9% among women. In terms of age, the percentage of smokers among people between 18 and 69 years old was 23.4% in 2018[4]. Moreover, it is estimated that smoking is involved in at least 13% of female infertility cases, as well as other unhealthy lifestyles, such as alcohol and environmental stressors, which negatively impact human health[5]. In addition, during the last few years, several alternatives to traditional cigarette smoking, such as electronic cigarettes (e-cig.) and heat-not-burn (HnB) products, have been widely spreading in Italy, especially among young men and women who want to stop cigarette smoking[6]. Today, it is estimated that 1.3 million Italians smoke electronic cigarettes and that 739,000 Italians have used HnB products, including 329,000 never smokers[7,8]. As smokers' consumption is still high in Italy, the average age of first pregnancy appears to be rising to around 31.2 years, due to socioeconomic developments. With the aging process, the ovarian reserve decreases leading to increased atretic follicles, lower oocyte quality, and lower oocyte recruitment during ovulation[9]. Indeed, smoking leads to alterations in the morphology of the oocyte meiotic maturation path caused by alteration of cellular oxidative balance, and the risk of ovarian reserve damage is higher as the number of cigarettes smoked daily and the exposure time grow[10]. Ovarian reserve is represented by antral follicle account (AFC) and anti-Müllerian hormone (AMH), the glycoprotein hormone produced by the granulosa cells of the pre-antral and small antral follicle, that has been assessed along with follicle-stimulating hormone (FSH), as an indicative marker of the ovarian reserve and fertility and a reliable marker for IVF results[11]. During the women's life, the secretion of AMH progressively decreases, as does the number of primordial follicles, and so on the ovarian antral follicle count. Among women performing IVF cycles, active smoking is related to lower live birth per cycle, lower odds of clinical pregnancy per cycle, and significantly higher odds of spontaneous miscarriage[12]. Our clinical study aimed to investigate the correlation between women's smoking habit and ovarian reserve, and IVF outcomes of intracytoplasmatic sperm injection (ICSI) technique in women performing controlled ovarian stimulation (COH). We also included in our analysis all the different typologies of smoking habits with the aim to sensitize young women who have access to IVF medical centers to avoid harmful lifestyle choices, such as smoking..

Patients and Methods

Between January 2019 and February 2022, at the Physiopathology of Reproduction and Andrology Unit, Sandro Pertini Hospital in Rome, in collaboration with the Obstetrics and Gynecology Unit of Santa Maria Goretti Hospital, Latina, a group of infertile women listed for ICSI cycles were enrolled, providing them with a questionnaire which investigated the consumption of all kinds of smoking typologies (cigarette, cigar, tobacco, electronic cigarette, heated tobacco products), the time since the patient had started any smoking activity and the daily amount. Women who currently smoked ≥10 cigarettes/day (or >10 e-cig./HnB products/day for at least one year) were considered and included in the study. Non-smoking women were those who did not smoke or had stopped for at least one year and who did not cohabit with smoking partners. Inclusion criteria: both partners' age ≤40 years, idiopathic or tubal infertility, body mass index (BMI) between 20 and 30 kg/m^2. Exclusion criteria: ongoing pregnancy, suspected anovulation and recurrent pregnancy loss, pelvic endometriosis, polycystic ovary syndrome, basal FSH >10 mUI/ml, chromosomal alterations, cardiovascular diseases, ovarian surgery, and current dual users' smokers and second-hand smoking exposure[13].

All women fulfilling the inclusion/exclusion criteria were examined at our reproductive center, with a close focus on their medical history and on smoking habits, which were investigated by the questionnaire given before the beginning of ICSI cycle. All women were examined by a pelvic ultrasound performed by expert clinicians, who aimed to evaluate AFC, defined as the sum of antral follicles in both ovaries measured between the 2nd and 5th day of the menstrual cycle. Serum concentrations of FSH and AMH were also measured prior to the IVF tech-

niques. All women enrolled were prospectively followed and performed a controlled ovarian stimulation (COH) with an antagonist protocol, and subsequent ICSI. Specifically, multipolar follicular growth was induced by antagonist protocol and stimulation with gonadotropins, starting from the 2nd day of the cycle. All patients were monitored starting from the 5th day of stimulation every two days, by measuring estradiol, progesterone, and ultrasound control. When the ultrasound and hormonal parameters were likely to be compatible with the oocyte maturity, the trigger was set by the administration of HCG 5,000/10,000 UI or triptorelin 0.2 mg/ml, and the ovarian pick-up (OPU) was performed after 32-36 hours from the trigger administration. One hour after decumulation, the oocytes were deemed suitable by our biologists' staff (oocytes in the MII stage) and were subjected to ICSI technique. Normal fertilization (fertilization rate - FR) was identified by the presence of two pronuclei (2PN) at the time of fertilization assessment, observed 16 to 19 hours after ICSI, using the invertoscope equipped with the Hoffman contrast system and with software for archiving images, according to the European Sociaty of Human Reproduction and Embryology (ESHRE) guidelines[14]. The outcomes of the study were confronting demographics and ICSI results between groups of smokers and non-smokers, and ICSI results between cigarette smokers *vs.* e-cigarette and HnB products smokers. Our observational prospective study respects the Helsinki declaration and was approved on 12/07/2019 by the ethical committee "Lazio 2" of our hospital ASL ROMA 2, number ID 49.19, protocol number: 0127710. Informed consent was obtained from all subjects involved in the research and all steps of the IVF patient program were conducted by our professional medical staff, and all biology laboratory analyses were performed by our IVF biologist staff.

Statistical Analysis

Statistical analysis was performed using unpaired tests when comparing groups, with the results expressed as mean±standard deviation (SD) and percentage. The unpaired Students' *t*-test was used to compare the average values of continuous variables such as age, BMI, hormone dosages, years of infertility, oocyte recovery parameters, and fertilization rate. All analyses were performed using the SAS software (release 9.4) (Milano, Italy). A p-value ≤ 0.05 was considered statistically significant.

Results

Out of the 490 infertile women admitted into the reproductive center during the studying period (2019-2022), 80 were excluded due to endocrinological alteration, such as polycystic ovarian or age >40. The remaining 410 women, fulfilling the inclusion/exclusion criteria, filled out the questionnaire regarding smoking activity and subsequently performed a controlled ovarian stimulation with gonadotropin and antagonistic protocol followed by ovarian pick-up and subsequent ICSI technique.

The smoking status of the patients was verified during the first stages of the IVF pathway to parenthood, through the submitted questionnaire. The answers given indicated that 203 women (49.5%) were active smokers, while 207 (51.5%) were non-smokers or declared that they had ended no more than a year ago. Out of the 203 active smokers, 103 (51%) were cigarette smokers, 60 (29%) were e-cigarette smokers and 40 (20%) were HnB products smokers. None of them were cigar smokers (Figure 1).

Age, BMI, basal FSH level, the total dose of gonadotropin given, years of infertility, and serum level of AMH (measured in ng/ml) were evaluated and confronted between the group of smokers and the group of non-smokers. Whilst all demographics and clinical features were comparable in both groups, it is remarkable that AMH was statistically lower in women who smoked, compared to non-smokers (all those parameters are shown in Table I).

Moreover, regarding IVF results, the non-smoking group reported a lower total dose of gonadotropin administered, a statistically higher total number of oocytes and MII oocytes retrieved, and a statistically higher FR when compared to smokers, as reported in Table II. Confronting tobacco cigarette smokers (subgroup A), with e-cig. smokers (subgroup B) and HnB products smokers (subgroup C), we did not detect statistically significant differences regarding ICSI results, such as the total number and number of mature (MII) stage oocytes retrieved. Only the number of germinal vesicles (GV) was statistically higher in subgroup B compared to A (p=0.04). Although FR values were slightly higher among e-cig smokers and HnB product users compared to cigarette users (70.56±3.05 and 70.16±4.15

in comparison to 68.12±2.21), those values were not statistically significant (Table III).

Discussion

Patient-oriented lifestyle has a significant impact on human health and fertility, both in men and in women, in particular on oocyte quality in women performing ICSI technique[15]. Cigarette smoke combustion produces a complex aerosol composed of about 4,000 chemicals, which contaminate the circulatory system and induce toxic damage reaching the target tissues, such as ovaries. This aerosol is characterized by a vapor phase and a particulate phase that contains toxic substances, such as polycyclic aromatic hydrocarbons benzopyrene, acenaphthylene, phenanthrene, pyrene, chrysene, and nitrosamines specific tobacco[16]. Low molecular weight hydrocarbons, such as benzene, butadiene, and toluene are mainly present in the vapor phase while hydrocyanic acid and ammonia are found in both phases. Those chemical substances increase the blood level of radical oxygen species (ROS) and oxidative and cellular stress, leading to alterations in biological tissues, cell damage, and death[17]. We performed a prospective analysis to figure out how smoking-induced damage can negatively impact ovarian reserve and ICSI ovarian outcomes. Cigarette smoking is associated with damage to folliculogenesis, steroidogenesis, and premature exhaustion of ovarian function: women who smoke enter menopause on average two years earlier than non-smokers[18]. In the animal model, an increase of ROS in the follicular fluid and granulosa cells has been demonstrated, with subsequent alteration of ovarian morphology and competence, due to alteration of oxidative balance, and irreversible damage of mitochondrial DNA[19]. Regarding hormonal results, in specific low AMH value in smokers compared to non-smokers (Table I), our study agrees with the work of Sansone et al[20], which reports that AMH value scored higher in non-smokers, in comparison with smoking patients with recurrent abortions. Indeed, the smoking habit links to damage to the cell structure and chemical stability, leading to the grafting of a chained radical process, which can damage the cells through the activity of ROS, leading to biochemical and structural damage both to the external and theca interna levels[21]. Likely AMH levels could be more sensitive to direct smoking toxic damage, compared to AFC: since the glycoprotein AMH is produced at the follicular level, any cell damage due to smoke results in lower levels of this glycoprotein, correlating with a reduction of AMH values, and so on the ovarian reserve[22]. In addition, our data regarding the total dose of

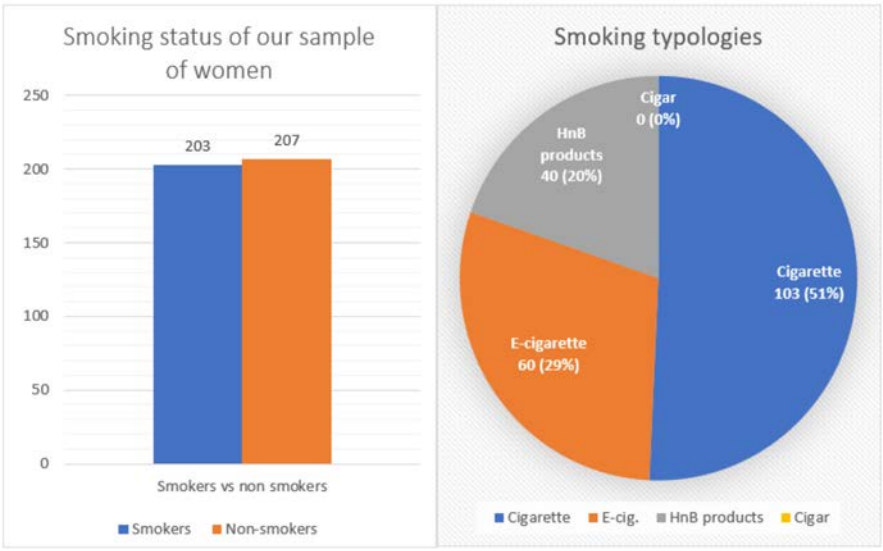

Figure 1. Column chart: graphical representation of smoking status of our sample of 410 women. Sequentially filling out the questionnaire, 203 (49.5%) women were defined as active smokers while 207 (51.5%) were defined non-smokers. Pie chart: of 203 smokers, 103 (51%) were cigarette smokers, 60 (29%) e-cig smokers, 40 (20%) HnB products smokers. No woman was a cigar smoker.

Table I. Patients' demographic and clinical features confronted between groups of smokers and non-smokers.

Demographics parameters n: 410 women	Smokers n: 203	Non-smokers n: 207	*p*-value
Age - years (SD)	36.9±2.4	38.1±3.4	ns
BMI kg/m^2 - mean (SD)	24.±2.9	23.7±1.8	ns
Duration of infertility, years - mean (SD)	3.1±1.1	3.7±2.1	ns
Tubal factor of infertility - n. (%)	106 (52.3)	102 (56.6)	ns
Idiopathic factor of infertility - n. (%)	97 (47.7)	80 (43.4)	ns
Basal FSH (mIU/ml) - mean (SD)	9.1±2.1	7.9±2.7	ns
AFC - mean (SD)	11.9±2.1	12.3±1.7	ns
AMH (ng/ml) - mean (SD)	1.3±2.3	2.1±4.5	<0.05
Days of stimulation - mean (SD)	10.5±4.5	9.8±2.4	ns
Total dose of gonadotropin (UI) - mean (SD)	1,850±860	1,730±780	<0.05

SD: standard deviation, n: number, AFC: antral follicle count; AMH: anti-Müllerian hormone, FSH: follicle-stimulating hormone, ns: not statically significant.

gonadotropin, which was slightly higher in the smokers' group, is consistent with the study of El-Nemr et al[23], which reported a significantly higher mean dosage of gonadotrophins for ovarian stimulation than the non-smokers (48.1±15.6 *vs.* 38.9±13.6 ampoules (75 IU/ampoule) $p<0.0001$. Regarding ICSI technique outcomes, our results reported a lower number of oocytes retrieved in the smokers' group with respect to non-smokers, and it is in line with those of previous studies[24-26]. Indeed, Gruber et al[24] demonstrated that smokers presented a higher number of non-fertilized oocytes than non-smokers, and according to the study Fuentes et al[25], we reported a lower number of oocytes retrieved in active smokers' groups in comparison with non-smokers. Furthermore, our results agree with the systematic review of Budani and Tiboni[26], in which authors reported that the number of oocytes retrieved decreased by 40% for smoking women that showed in their lifetime had adjusted risks of 2.71 of not achieving a pregnancy and 2.51 of not having a live birth deliver. Indeed, in smokers' retrieved oocytes an increase of the zona pellucida thickness happens, and this condition is related to a major difficulty to fertilize[27]. Individual sensitivity, dose, time, and type of exposure also play a role in the impact of smoke constituents on human fertility, and IVF represents an interesting model for appreciating the toxic effects of smoking substances on human gametes. As reported by Dechanet et al[28], all stages of reproductive function, such as folliculogenesis, steroidogenesis, embryo transportation, endometrial receptivity, endometrial angiogenesis, and uterine blood flow are targets for cigarette smoke components and especially the ovarian reserve. Indeed, our result observed a higher empty zona pellucida (EZP) oocyte number in smokers, in comparison with non-smokers, revealing a decreased oocyte quality, and poor ovarian response

Table II. PIVF outcomes, in specific oocyte quality and quantity, confronted between group of smokers (including cigarettes, electronic cigarettes-e-cigarettes and heat-not burn-HnB-products) and non-smokers.

IVF results: Oocyte parameters n: 410 women	Smokers group n: 203	Non-smokers group n: 207	*p*-value
Number of cigarette/e-cig./HnB products smoked per day - mean (SD)	12±2.3	/	/
years of smoking activity - mean (SD)	12.4±3.2	/	/
n° total oocyte retrieved/patient - mean (SD)	5.21±0.9	6.55±3.5	<0.001
n° MII stage/patient - mean (SD)	4.2±1	4.4±1.1	ns
n° MI stage/patient - mean (SD)	0.4±0.8	0.7±1.1	ns
n° GV /patient - mean (SD)	0.4±0.2	0.3±0.1	ns
n° EZP/patient - mean (SD)	0.51±0.1	0.2±0.1	<0.05
n° atresic oocyte/patient - mean (SD)	0.1±0.1	0.04±0.02	ns
Fertilization rate - % (SD)	68.12±2.21	72.16±3.05	0.03

SD: standard deviation, n: number, AFC: antral follicle count; AMH: anti-Müllerian hormone, FSH: follicle-stimulating hormone, ns: not statically significative.

Table III. IVF outcomes confronted between group of cigarette smokers (subgroup A) *vs*. electronic cigarette (e-cig.) smokers (subgroup B) and *vs*. heat-not-burn (HnB) products smokers' groups (subgroup C).

Smoking women n: 203	Cigarette smokers (A) n: 103	E-cigarette smokers (B) n: 60	HnB products smokers (C) n: 40	*p*-value A *vs.* B A *vs.* C
Number of cigarette/e-cig./HnB products smoked per day - mean (SD)	11±1.3	10.5±1.8	10.3±1.2	ns ns
years of smoking activity - mean (SD)	12±2.2	11±1.8	10.4±2.2	ns ns
n° total oocyte retrieved/patient - mean (SD)	4.98±0.9	5.11±3.5	5.06±3.5	ns ns
n° MII stage/patient - mean (SD)	4.2±1.3	4.3±1.1	4.25±1.6	ns ns
n° MI stage/patient - mean (SD)	0.5±0.8	0.47±1.1	0.35±1.1	ns ns
n° GV /patient - mean (SD)	0.33±0.2	0.48±0.1	0.4±0.1	0.04 ns
n° EZP/patient - mean (SD)	0.3±0.1	0.22±0.1	0.3±0.5	ns ns
n° atresic oocyte/patient - mean (SD)	0.11±0.1	0.18±0.02	0.1±0.2	ns ns
Fertilization rate - % (SD)	68.12±2.21	70.56±3.05	70.16±4.15	ns ns

SD: standard deviation, n: numbers, M: metaphase, EZP: empty zona pellucida, GV: germinal vesicles, ns: not statically significant.

in the smokers' group[29]. Additionally, our result highlighted that FR was statistically lower in the smokers' group (68.12 *vs.* 72.16%) (Table II), and this result is consistent with the study by Wesselink et al[30], in which authors associated smoking with small reductions in fecundability, in women who smoked ≥10 cigarettes/day for ≥10 years. Among other types of smoking habits, the consumption of electronic cigarettes has increased during the last year in our country, especially among former cigarette smokers. E-cigarette consumption, even the nicotine-free ones, contains many harmful substances including nicotine, ultrafine particles, heavy metals, polycyclic aromatic hydrocarbons, and volatile organic compounds, which act as endocrine disruptors. Indeed, the clinical effect of those vaping devices, both with and without nicotine, seems to be dangerous for pregnancy conditions[31]. As a matter of fact, e-cigarettes cannot be considered a completely healthy alternative to smoking, and even though there is limited evidence so far[32], vaping has been linked to deleterious effects on the human reproductive system. A recent study[33] reports a slightly reduced fecundability but estimates of its independent and joint associations with combustible cigarette smoking are inconsistent and imprecise. Moreover, adverse health effects related to e-cigarette aerosol exposition are influenced by several factors, including e-liquid components, physical device factors, chemical changes related to heating, and the health of e-cigarette users[34]. There is also limited data regarding the consumption of HnB products and their correlation with human fertility. Compared to conventional tobacco cigarettes, HnB devices reduce customers' exposure to hazardous and potentially hazardous constituents, and for this reason, HnB products seem to be an alternative option for smokers. In addition, HnB products do not eliminate the probability of tobacco-related diseases' development, caused by the toxic substances produced by vaporization, although in a reduced quantity compared to cigarettes[35], as lower concentrations of

tar, carbonyls, carbonium monoxide free radicals, and nitrosamines[36]. In specific HnB products devices heat tobacco to temperatures of 250-350°C, depending on the device allowing for the volatilization of nicotine and flavorings, while potentially limiting the production of combustion-related toxicants, with a lower level of free radicals, like e-cig, when compared to conventional cigarettes[37]. Through a prospective analysis, we appreciated similar results among women afferent to our reproductive center, regarding the number and quality of oocytes retrieved, confronting tobacco smokers *vs.* e-cig and HnB product users (Table III). Only the number of GV oocytes was statistically higher in e-cigarette smokers compared to cigarette smokers. Interestingly, as described in the result chapter, FR was not statistically different in those three groups of smokers. Those results might be due to the possibility that all kinds of smoking activity may increase the level of ROS, and in specific of nicotine metabolite as cotinine, causing a direct or indirect impact on the female's reproductive system, and so on lower IVF results, in childbearing women performing ICSI technique[38]. It is plausible that numerous harmful substances produced by the combustion of those devices, such as metals, organic compounds, aldehydes, and formaldehyde, which is particularly harmful to human health, could interfere with the female reproductive system, damaging ovarian function and alternating folliculogenesis and oocyte quantity and competence[39]. To our knowledge, this is the first study that investigates the impact of different typologies of smoking, such as e-cigarettes and HnB products, in infertile women performing ICSI cycles, and confronting those different typologies of smoking and clinical results of IVF cycles. The strength of this study is to exclude smoking male partners, eliminating a confounding factor represented by women exposed to second-hand tobacco smoke, which might reduce the IVF clinical success, describes our results only up to oocyte fertilization, to focus our analysis on oocyte features and ICSI technique[40]. Furthermore, we chose to exclude both women and respective partners > 40 years, because of the impact of age on exhaustion on ovarian reserve and seminal parameters, and so on reproductive outcomes in IVF stimulation programs[41,42]. Additionally, we thought it was topical to include different kinds of smoking habits, such as the consumption of e-cigarettes and HnB tobacco products, that are widely spreading in our country.

Limitations of our study are the lack of randomization and of biochemicals smoking markers measurements, e.g., in the follicular fluid, as nicotine-derived product as cotinine, the limited sample of women included, and a bias represented by the second-hand smoke exposition among the male partners of women who currently smoke. Moreover, physical exercise, alcohol drinking, caffeine consumption, diet, and diet supplement consumption, were not taken into consideration in our analysis.

Finally, it is crucial to continue ongoing research on the potentially harmful effects of e-cigarettes, HnB products and other typologies of smoking, on maternal and paternal reproductive health, and on fetal health and development. Not less important, clinicians should adequately educate women and men who desire pregnancy, to avoid, or at least reduce, exposure to pollutants, such as those derived from the combustion of tobacco and its derivatives. Further studies will need to investigate different toxicology mechanisms underlying different typologies of smoking, such as e-cigarettes and HnB products.

Conclusions

Smoking should be viewed as a significant issue that heavily affects our society, and one of the most relevant factors that jeopardize human fertility, especially the ovarian reserve. During the last few years, different typologies of smoking are spreading in our country among women and men of childbearing age. Tobacco cigarette consumption reduces ovarian reserve and negatively impacts IVF results of controlled ovarian stimulation, through an increase in ROS production. While still little is known regarding the possible impact of the electronic cigarette and the HnB reproductive system, our results link to a possible negative impact on oocyte parameters in ICSI cycles. Further clinical studies are needed, based on the numbers of women and appropriate estimation of tobacco exposure, expanding the clinical research field to all different types of smoking, such as e-cigarettes and-HnB products, thus expanding our knowledge on molecular and toxicology pathways that may impact the reproductive system. As clinicians, even more for those involved in the IVF field, fertility preservation represents our primary goal. We all must encourage women and men to embrace healthier lifestyles and guide them towards integrative therapeutic options aimed at

reducing oxidative stress, especially those caused by the combustion of tobacco and other smoking devices.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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Informed Consent
Informed consent was obtained from all patients involved in this study.

Ethics Approval
This study was conducted following the Ethical Principles of the Helsinki Declaration and national laws. The present study was approved by the Ethical Committee "Lazio 2" of the ASL ROMA 2, on 02/07/2019, protocol number: 0127710, study ID: 49.19.

Data Availability
The data presented in this study are available upon request from the corresponding author. The data are not publicly available due to privacy issues.

Authors' Contributions
FG wrote and edited the manuscript. All other authors made an equal contribution to the realization of this study. All the authors read and agreed to the current published version of the manuscript.

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