Reproductive Sciences (2021) 28:37–42
https://doi.org/10.1007/s43032-020-00282-8

REPRODUCTIVE ENDOCRINOLOGY: ORIGINAL ARTICLE

Waterpipe Tobacco Smoke Exposure during Lactation—Susceptibility of Reproductive Hormones and Oxidative Stress Parameters in Male Progeny Rats

Nour A. Al-Sawalha[1] · Indira D. Pokkunuri[2] · Karem H. Alzoubi[1] · Omar F. Khabour[3] · Bashar N. Almomani[4]

Received: 15 May 2020 / Accepted: 29 July 2020 / Published online: 3 August 2020

Abstract

There is a growing evidence for the public health hazards associated with waterpipe tobacco smoking (WTS). While the adverse effects of WTS exposure during pregnancy on the offspring are widely reported, its impact during breastfeeding remains less understood. The effects of WTS exposure during lactation on the reproductive hormones and oxidative stress biomarkers of adult male progeny were examined. Lactating rats received either fresh air (controls) or mainstream WTS for 1 h twice/day from day 4 to day 21 of lactation. The offspring was then followed up until week 20. The data indicated that WTS exposure in the lactating animals reduced the levels of follicle-stimulating hormone (FSH), prolactin ($P < 0.05$), luteinizing hormone (LH) ($P = 0.1146$), and estradiol ($P = 0.0773$) in the blood in male progeny. While the activities of testicular superoxide dismutase (SOD), glutathione peroxidase (GPx) and the levels of thiobarbituric acid reactive substances (TBARS) and blood levels of testosterone ($P > 0.05$) remained unaltered, the activity of catalase increased significantly indicating an increased oxidant load in the WTS exposed rats compared to the controls. WTS exposure during lactation impairs male reproductive hormonal profile, augments oxidative damage, and potentially affects male fertility in male offspring rats.

Keywords Waterpipe smoking · Reproductive hormones · Oxidative stress · Lactation · Male progeny

Introduction

Waterpipe tobacco smoke (WTS), where tobacco smoke is passed through water prior to inhalation, became as prevalent as cigarette tobacco use [1], especially among youth and women [2]. It was estimated that about 100 million people use waterpipe daily [3]. The increased popularity of WTS is due to the misconception of its reduced health hazard compared with cigarette smoke. Reports suggest that the prevalence rates for WTS during pregnancy ranged from 5 to 9% in Middle East and North Africa (MENA) countries such as Lebanon, Iran, and Jordan [4–6]. Examining the composition of waterpipe smoke revealed the presence of nicotine, heavy metals, tar [7], carbon monoxide [8], and polycyclic aromatic hydrocarbons [9, 10]. There is an urgent need for a critical evaluation of the adverse effects of WTS, especially in pregnant and lactating women where the implications are passed onto the offspring.

The harmful effects of waterpipe smoking during pregnancy on the newborns are widely reported. For example, it resulted in low birth weight [11, 12], low anthropometric measurements; newborn head circumference and length [12], increased risk of infant mortality [13], and pulmonary complications at birth [14]. Our previous studies showed that prenatal exposure to WTS led to an imbalance in reproductive hormones and increased oxidative damage in adult male offspring rats [15]. Also, WTS represents a growing tobacco epidemic and there is an increase in the proportion of women who resume smoking waterpipe during the lactation stage resulting in raising the health risk of the breastfed offspring.

✉ Nour A. Al-Sawalha
nasawalha@just.edu.jo

[1] Department of Clinical Pharmacy, Faculty of Pharmacy, Jordan University of Science and Technology, P.O. Box 3030, Irbid 22110, Jordan

[2] College of Pharmacy, University of Houston, Houston, TX 77204, USA

[3] Faculty of Applied Medical Sciences, Jordan University of Science and Technology, Irbid, Jordan

[4] Ministry of Health, National Institute of Forensic Medicine, Amman, Jordan

Male infertility has become a universal concern. Reports suggest about 30 million men to be infertile worldwide, contributing overall to 50% of the infertile couples [16]. While there are several causes for male infertility, sperm disorders are considered as critical factors. Incidentally, studies revealed an association between WTS in males and lower sperm count albeit with normal morphology but with higher levels of FSH, LH, and testosterone [17]. Ali and colleagues reported impaired levels of reproductive hormones, altered oxidative status [18, 19], and reduced spermatogenesis [19] in the offspring of the mice that were exposed to WTS. Previous data showed that exposure to nicotine during pregnancy and lactation led to altered levels of FSH and LH [20], reduced sperm motility, and induced DNA fragmentation of sperms [21] in adult male offspring rats, but the exclusive effects of WTS remain elusive. The goal of the current study was to examine the effect of WTS exposure during lactation on the reproductive hormones and oxidative damage in testis in adult male progeny rats.

Methods

Animals

Adult Wistar rats (9–10 weeks old), male and female ($n = 22$ each), were purchased from the Animal Care Unit at Jordan University of Science and Technology. The animals were maintained under constant room temperature (24 ± 1 °C), were given free access to food and water, and were maintained in 12:12 light-dark cycles. Animals were mated by housing one male and one female rat in the same cage for a night, and the next morning, pregnancy was detected by checking the vaginal plug [22]. Pregnant rats were provided with free access to water and food, were housed separately, and were followed up until delivery. After delivery, 22 lactating dams were randomly divided into two groups, one group received fresh air (control) and the other received WTS. All procedures were performed in accordance with the Animal Care and Use Committee (ACUC) of Jordan University of Science and Technology.

Mainstream WTS Exposure

A whole-body exposure system was used for the rats in the WTS group to expose them to mainstream WTS twice/day for 1 h each from days 4 to 21 of lactation [23] since it is important not to separate letters from dams for the first 4 days [24]. Briefly, the exposure system is composed of a whole-body exposure chamber attached to a waterpipe smoking machinery, where the smoke is withdrawn from the diaphragm pump. The Beirut method was employed to program the smoking machine for the exposure procedure (171 puffs of 2.6-s duration with 17-s duration in between the puffs) to mimic the real situation [25]. About 10 g of Nakhla Double Apple tobacco was used in each exposure. Carbon monoxide (CO) analyzer (Monoxor II, Bacharach Inc.) was used to ensure consistent levels of CO exposure to all animals (1050 ± 100 ppm, mean ± SD). The litters were separated from the dams during the exposure sessions [24]. Male pups (one per dam) were monitored until week 20.

Measurement of Male Reproductive Hormones in the Blood Plasma

The litters, 20 weeks old, were sacrificed in the morning to reduce the diurnal variations of the hormones in the blood [26]. The plasma was separated from blood by centrifuging the latter for 10 min at 2500 rpm at room temperature in EDTA tubes. The collected plasma was stored at − 80 °C until further analysis. Enzyme-linked immunosorbent assay (ELISA)–based methods were used to measure the plasma concentrations of FSH, LH, and prolactin (Abbexa, Cambridge, UK), as well as estradiol and testosterone (Demeditec Diagnostics GmbH, Germany), following the manufacturer's instructions.

Measurement of Oxidative Damage Parameters and Testosterone in the Testis

The testes were isolated, washed with cold PBS, and snap frozen in liquid nitrogen before storing them at − 80 °C until further processing as described previously [15]. Stored testes were homogenized, and protein concentration was determined by using the BioRAD kit (Hercules, CA, USA). In the homogenates, the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) (both from Sigma-Aldrich Corp., MI, USA) catalase (Cayman Chemical, MI, USA), and the concentration of thiobarbituric acid–reactive substances (TBARS) (Cayman Chemical, MI, USA) were measured by kit-based methods following the manufacturers' instructions. The absorbance at the appropriate wavelengths was measured by using a microplate reader (BioTek, Winooski, VT, USA).

Statistics

All the statistical analyses were performed using GraphPad Prism 4®. Data were presented as mean ± SEM. Normal distribution in the data was determined by using Shapiro–Wilk and D'Agostino and Pearson omnibus normality tests, and the analyses were carried out by using Student's t test or the Mann–Whitney test; $P < 0.05$ was considered statistically significant.

Results

Effects on FSH, LH, and Prolactin Levels

The circulatory levels of FSH (ng/ml: 32.24 ± 4.0 in WTS vs 16.92 ± 2.4 in control, $P = 0.0034$) and prolactin (ng/ml: 4.22 ± 0.7 in WTS vs 8.02 ± 0.6 in control, $P = 0.0010$) were significantly reduced in adult male offspring of the rats exposed to WTS during lactation compared with fresh air–exposed controls (Fig. 1a and c). The circulatory levels of LH were found to be slightly reduced, although not significant, in the rats exposed to WTS during lactation (mIU/ml: 33.59 ± 5.8 in WTS vs 47.48 ± 5.9 in control, $P = 0.1146$) (Fig. 1b).

Effects on Male Sexual Hormone Levels

The circulatory levels of testosterone in the adult male progeny of the rats exposed to WTS during lactation remained unaltered compared with those in the control (pmol/l: 26.91 ± 9.3 in WTS vs 25.35 ± 5.3 in control, $P = 0.5148$) (Fig. 2a). However, in the male progeny of the rats exposed to WTS during lactation, a trend of reduced estradiol in the offspring rats was observed, although it was not significant (pg/ml: 1.07 ± 0.4 in WTS vs 2.72 ± 0.6 in control, $P = 0.0773$) (Fig. 2b).

Effects on Testicular Oxidative Stress Parameters

No significant changes were observed in the activities of SOD (units/mg protein: 0.72 ± 0.2 in WTS vs 0.72 ± 0.1 in control, $P = 0.9841$) or GPx enzymes (units/mg protein: 0.27 ± 0.04 in WTS vs 0.30 ± 0.02 in control, $P = 0.5012$) in the testes of adult male offspring of the rats exposed to WTS during lactation (Fig. 3a and b). However, the activity of catalase enzyme was significantly increased by WTS exposure during lactation in testicular tissue of offspring rats (units/mg protein 9.00 ± 1.2 in WTS vs 2.57 ± 0.6 in control, $P < 0.0001$) (Fig. 3c). The levels of TBARS were not affected in the testes of adult male offspring of the rats exposed to WTS during lactation (μM/mg protein: 0.70 ± 0.05 in WTS vs 0.69 ± 0.10 in control, $P = 0.9011$) (Fig. 3d).

Discussion

WTS represents a growing global tobacco epidemic. Current study reveals the detrimental effects of WTS exposure exclusively during the lactation period on the reproductive hormones and oxidative damage parameters in adult male progeny animals. The study showed a reduction in the plasma levels of FSH and prolactin, but not testosterone, associated with a tendency of reduced LH and estradiol levels, in the offspring rats that were exposed to WTS during lactation. Also, the progeny of the rats exposed to WTS during lactation showed a significant increase in catalase activity and unaltered activities of testicular SOD, GPx and TBARS levels. To our knowledge, this is the first time the effects of WTS exposure exclusively during lactation on male fertility hormones and oxidative damage parameters were studied.

The regulation of reproductive axis begins at hypothalamus, which secrets gonadotropin-releasing hormone (GnRH), which in turn stimulates the secretion of LH and FSH hormones from the anterior pituitary. LH and FSH control the gonadal function; LH induces the release of testosterone from Leydig cells of the testes [27], and FSH is involved in spermatogenesis in Sertoli cells, along with testosterone and inhibin hormones [27–29]. Prolactin, another hormone

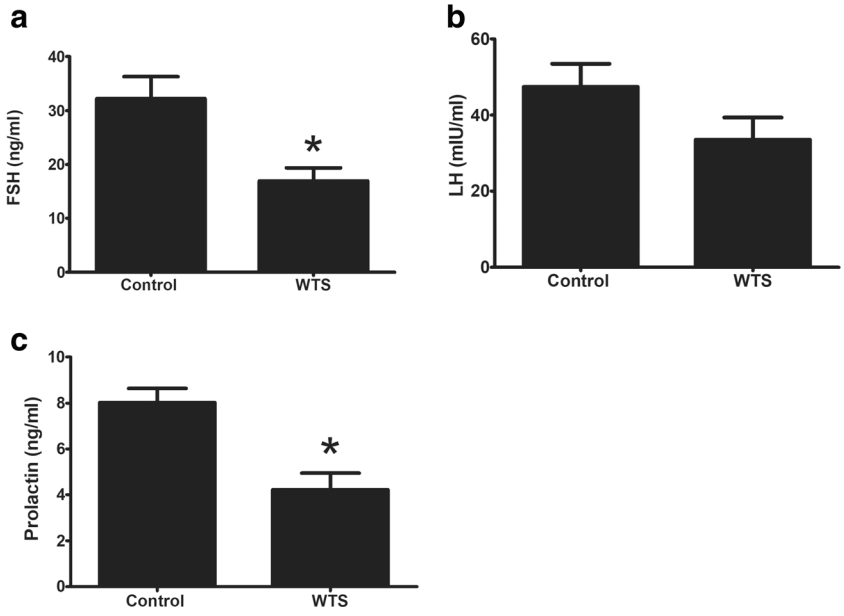

Fig. 1 Effect of WTS exposure during lactation on circulatory levels of FSH, LH, and prolactin in adult male offspring rats. Lactating rat dams were exposed to either fresh air (control) or WTS during lactation. Adult male offspring rats, 20 weeks old, were sacrificed, and circulatory levels of **a** FSH, **b** LH, and **c** prolactin were measured. Values represent the mean ± SEM of data from 9 to 11 adult male offspring rats in each group. $P < 0.05$ was considered statistically significant. * indicates significant difference from the control group

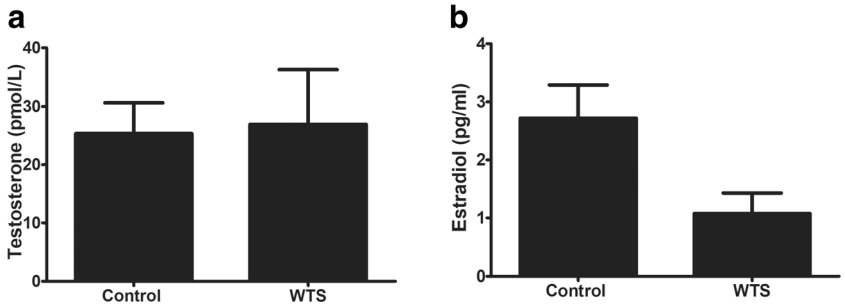

Fig. 2 Effect of WTS exposure during lactation on circulatory levels of testosterone and estradiol in adult male offspring rats. Lactating rat dams were exposed to either fresh air (control) or WTS during lactation. Adult male offspring rats, 20 weeks old, were sacrificed and circulatory levels of **a** testosterone and **b** estradiol were measured. Values represent the mean ± SEM of data from 9 to 11 adult male offspring rats in each group. $P < 0.05$ was considered statistically significant

released from the pituitary gland, is also involved in male fertility by stimulating the testicular function and increasing the density of LH and FSH receptors [30]. In the current study, there was a significant decrease in FSH and prolactin levels and a non-significant but steady decrease in LH levels in the male progeny of the rats that were exposed to WTS during lactation. The current results are consistent with Weisberg findings, where nicotine exposure altered hypothalamic–pituitary axis by stimulating the release of cortisol, growth hormones, vasopressin, and oxytocin that in turn reduced the secretion of LH and prolactin [31]. Furthermore, Ochedalski and colleagues reported reduced circulating levels of LH, FSH, and prolactin in smokers than in non-smokers [32]. Low level of prolactin in males is critical as it is associated with infertility, reduced seminal vesicles' function, and psychological disorders [33]. Future studies should examine the levels of gonadotropin-releasing hormone as well as the modulators of prolactin release as dopamine in offspring rats after WTS exposure during lactation.

Testosterone has a significant role in the sexual characteristics and function of males as well as spermatogenesis [34]. The current study did not show significant alterations in the circulating level of testosterone in the adult male offspring of the dams exposed to WTS during lactation. Consistent finding was observed by other studies with active smoking, where the levels of testosterone were not altered in smokers compared with non-smokers [32, 35].

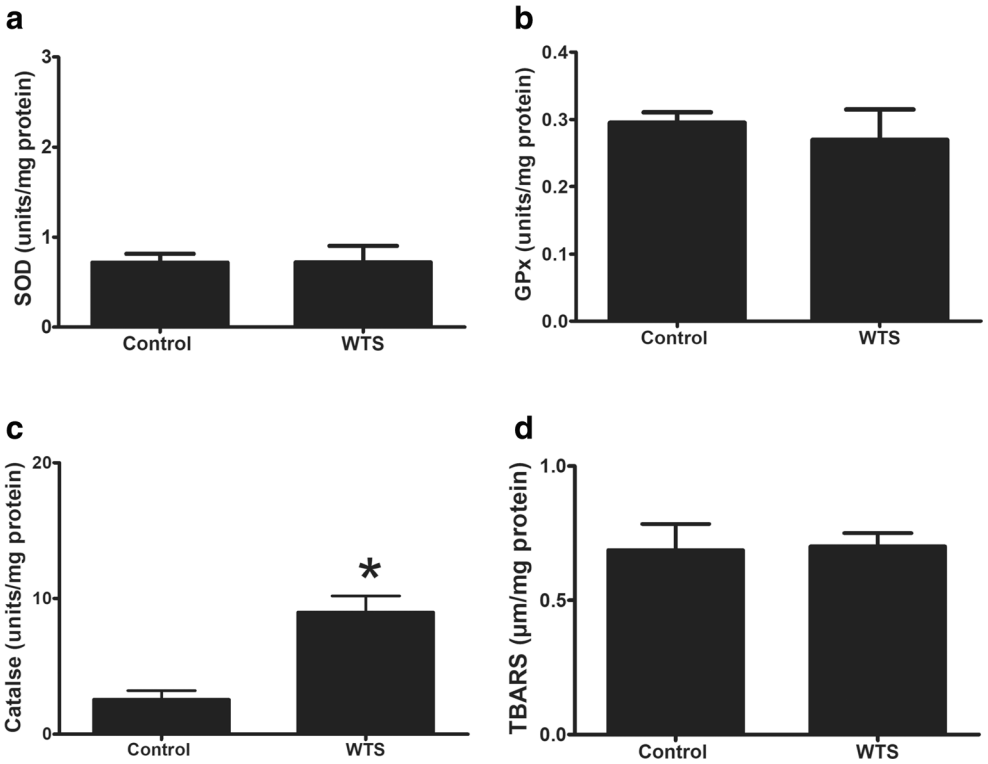

Fig. 3 Effect of WTS exposure during lactation on oxidative stress biomarkers in testes of adult male offspring rats. Lactating rat dams were exposed to either fresh air (control) or WTS during lactation. Adult male offspring rats, 20 weeks old, were sacrificed; testes were homogenized; and the activity of **a** SOD, **b** GPx, and **c** catalase, and levels of **d** TBARS were measured. Values represent the mean ± SEM of data from 9 to 11 adult male offspring rats in each group. $P < 0.05$ was considered statistically significant. * indicates significant difference from the control group

Estradiol plays an important role in modulating spermatogenesis, libido, and erectile function [36]. Current study showed reduced levels of plasma estradiol in adult male progeny of the rats exposed to WTS during lactation. The data is in coherence with that of Ali and colleagues, where chronic exposure (for 6 months) to WTS resulted in decreased plasma concentration of estradiol in mice [19]. Inconsistent findings were observed in the levels of estrogen and testosterone with active WTS exposure in mice for 1 month [18] and prenatal exposure to WTS [15]. The differences in the time of WTS exposure, and the source of measurement, blood vs tissue, could explain this discrepancy.

The reduced levels of FSH and prolactin associated with tendency of reduced LH and estradiol led to an alteration of the hypothalamic–pituitary–gonadotropin (HPG) axis in adult male offspring of the WTS-exposed rats during lactation. However, this alteration was persistent although the exposure to WTS was stopped. This could be explained by possible epigenetic modifications in the genes that are involved in regulating the HPG axis. Future studies should examine the morphological and functional alterations in different testicular cells such as Leydig and Sertoli cells as well as the morphological features of sperm.

Previous studies in animal models reported testicular oxidative stress upon WTS exposure. For example, a 6-month chronic exposure to WTS in adult male mice reduced the activities of several antioxidant enzymes, specifically glutathione reductase and catalase, while increasing the activity of SOD [19]. However, exposure to WTS for 1 month reduced the activity of SOD, catalase, and glutathione reductase [18]. In the current study, we found an increase in the testicular catalase activity, but no alteration was detected in the activities of SOD and GPx or the level of TBARS, a lipid peroxidation end product, in the offspring of the rats subjected to WTS during lactation. Prenatal exposure to WTS increased the activity of catalase in the testes of offspring rats, consistent with the current finding [15]. The increased catalase activity indicates a mechanism to compensate the increased oxidant load by WTS exposure. Increased oxidative stress leads eventually to male infertility [37, 38]. Future studies should examine the activity of other antioxidants such as glutathione reductase and the levels of reactive oxygen species (ROS) such as hydrogen peroxide among other oxidants.

There are a few limitations in the current study. First of all, the levels of nicotine or any major metabolite of nicotine, such as cotinine, were not measured here due to technical limitations. Second, neither the exact composition nor the specific component of WTS that caused the effects revealed in the current study is known. However, human subjects are exposed to all components of the WTS and not to only a single one. Third, the molecular mechanism of the epigenetic alteration was not examined in the current study. Fourth, the fertility assessment was not evaluated.

Conclusions

In conclusion, the current study showed that WTS exposure during lactation alters the male reproductive hormonal profile and disturbs oxidative balance in the male offspring rats. These alterations might affect the fertility negatively. These results imply a critical need for strict guidelines to impede waterpipe smoking during the breastfeeding period.

Acknowledgments The authors thank Weam Alyacoub, BSc and Laila Abo Haweileh, MSc for their technical assessment.

Authors' Contributions NAL performed the research and wrote the draft; IDP, KHA, OFK, and BNA interpret the data and critically revised the manuscript; all authors approved the submitted version.

Funding Information The financial support for this research was provided by the Deanship of Research at Jordan University of Science and Technology (grant numbers 70/2019).

Compliance with Ethical Standards The animal procedure was approved by the Animal Care and Use Committee (ACUC) at JUST.

Competing Interests The authors declare that they have no competing interests.

Disclaimer The funding agency has no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Abbreviations *ACUC,* Animal Care and Use Committee; *CO,* carbon monoxide; *ELISA,* enzyme-linked immunosorbent assay; *FSH,* follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *GPx,* glutathione peroxidase; *HPG,* hypothalamic–pituitary–gonadotropin; *LH,* luteinizing hormone; *MENA,* Middle East and North Africa; *SOD,* superoxide dismutase; *TBARS,* thiobarbituric acid–reactive substances; *WTS,* waterpipe tobacco smoking

References

1. Akl EA, Gunukula SK, Aleem S, Obeid R, Jaoude PA, Honeine R, et al. The prevalence of waterpipe tobacco smoking among the general and specific populations: a systematic review. BMC Public Health. 2011;11:244.
2. Jawad M, Charide R, Waziry R, Darzi A, Ballout RA, Akl EA. The prevalence and trends of waterpipe tobacco smoking: a systematic review. PLoS One. 2018;13(2):e0192191.
3. Wolfram RM, Chehne F, Oguogho A, Sinzinger H. Narghile (water pipe) smoking influences platelet function and (iso-)eicosanoids. Life Sci. 2003;74(1):47–53.
4. Azab M, Khabour OF, Alzoubi KH, Anabtawi MM, Quttina M, Khader Y, et al. Exposure of pregnant women to waterpipe and cigarette smoke. Nicotine Tob Res. 2013;15(1):231–7.
5. Mirahmadizadeh A, Nakhaee N. Prevalence of waterpipe smoking among rural pregnant women in southern Iran. Med Princ Pract. 2008;17(6):435–9.
6. Chaaya M, Jabbour S, el-Roueiheb Z, Chemaitelly H. Knowledge, attitudes, and practices of argileh (water pipe or hubble-bubble) and cigarette smoking among pregnant women in Lebanon. Addict Behav. 2004;29(9):1821–31.

7. Shihadeh A. Investigation of mainstream smoke aerosol of the argileh water pipe. Food Chem Toxicol. 2003;41(1): 143–52.
8. Cobb C, Ward KD, Maziak W, Shihadeh AL, Eissenberg T. Waterpipe tobacco smoking: an emerging health crisis in the United States. Am J Health Behav. 2010;34(3):275–85.
9. Sepetdjian E, Shihadeh A, Saliba NA. Measurement of 16 polycyclic aromatic hydrocarbons in narghile waterpipe tobacco smoke. Food Chem Toxicol. 2008;46(5):1582–90.
10. Jawad M, Eissenberg T, Salman R, Soule E, Alzoubi KH, Khabour OF, et al. Toxicant inhalation among singleton waterpipe tobacco users in natural settings. Tob Control. 2019;28(2):181–8.
11. Nematollahi S, Mansournia MA. The effects of water-pipe smoking on birth weight: a population-based prospective cohort study in southern Iran. 2018;**40**:e2018008.
12. Al-Sheyab NA, et al. Anthropometric measurements of newborns of women who smoke waterpipe during pregnancy: a comparative retrospective design. Inhal Toxicol. 2016;28(13):629–35.
13. Singh PN, et al. Maternal use of cigarettes, pipes, and smokeless tobacco associated with higher infant mortality rates in Cambodia. Asia Pac J Public Health. 2013;**25**(5_suppl):64S–74S.
14. Rachidi S, et al. Risky substance exposure during pregnancy: a pilot study from Lebanese mothers. Drug Healthc Patient Saf. 2013;5: 123–31.
15. Al-Sawalha NA, Almahmmod YM, Alzoubi KH Influence of prenatal waterpipe tobacco smoke exposure on reproductive hormones and oxidative stress of adult male offspring rats. 2019. 51(8): p. e13318.
16. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol. 2015;13:37.
17. Irene A, Fawzy NNKaAMA. Reproductive toxicity of tobacco shisha smoking on semen parameters and hormones levels among adult Egyptian men. Res J Environ Toxicol. 2011;5(5):282–92.
18. Ali BH, Adham SA, al Balushi KA, Shalaby A, Waly MI, Manoj P, et al. Reproductive toxicity to male mice of nose only exposure to water-pipe smoke. Cell Physiol Biochem. 2015;35(1):29–37.
19. Ali BH, et al. Chronic water-pipe smoke exposure induces injurious effects to reproductive system in male mice. Front Physiol. 2017;8: 158.
20. Paccola CC, Miraglia SM. Prenatal and lactation nicotine exposure affects Sertoli cell and gonadotropin levels in rats. Reproduction. 2016;151(2):117–33.
21. Miranda-Spooner M, Paccola CC, Neves FMO, de Oliva SU, Miraglia SM. Late reproductive analysis in rat male offspring exposed to nicotine during pregnancy and lactation. Andrology. 2016;4(2):218–31.
22. Al-Sawalha N, et al. Effect of prenatal exposure to waterpipe tobacco smoke on learning and memory of adult offspring rats. Nicotine Tob Res. 2018;20(4):508–14.
23. Al-Sawalha NA, et al. Effect of waterpipe tobacco smoke exposure during lactation on learning and memory of offspring rats: role of oxidative stress. Life Sci. 2019;227:58–63.
24. Gaworski CL, et al. In utero and lactation exposure of rats to 1R4F reference cigarette mainstream smoke: effect on prenatal and postnatal development. Toxicol Sci. 2004;79(1):157–69.
25. Katurji M, Daher N, Sheheitli H, Saleh R, Shihadeh A. Direct measurement of toxicants inhaled by water pipe users in the natural environment using a real-time in situ sampling technique. Inhal Toxicol. 2010;22(13):1101–9.
26. Oyeyipo IP, Raji Y, Bolarinwa AF. Nicotine alters male reproductive hormones in male albino rats: the role of cessation. J Hum Reprod Sci. 2013;6(1):40–4.
27. Griffin JE Disorders of the testes and male reproduction tract. Williams textbook of endocrinology, 1985.
28. Simoni M, Weinbauer GF, Gromoll J, Nieschlag E. Role of FSH in male gonadal function. Ann Endocrinol. 1999;60(2):102–6.
29. Majzoub A, Sabanegh E Jr. Testosterone replacement in the infertile man. Transl Androl Urol. 2016;5(6):859–65.
30. Gill-Sharma MK. Prolactin and male fertility: the long and short feedback regulation. Int J Endocrinol. 2009;2009:13.
31. Weisberg E. Smoking and reproductive health. Clin Reprod Fertil. 1985;3(3):175–86.
32. Ochedalski T, Lachowicz-Ochedalska A, Dec W, Czechowski B. Examining the effects of tobacco smoking on levels of certain hormones in serum of young men. Ginekol Pol. 1994;65(2):87–93.
33. Rastrelli G, Corona G, Maggi M. The role of prolactin in andrology: what is new? Rev Endocr Metab Disord. 2015;16(3):233–48.
34. Gannon JR, Walsh TJ. Testosterone and sexual function. Urol Clin North Am. 2016;43(2):217–22.
35. Richthoff J, Elzanaty S, Rylander L, Hagmar L, Giwercman A. Association between tobacco exposure and reproductive parameters in adolescent males. Int J Androl. 2008;31(1):31–9.
36. Schulster M, Bernie AM, Ramasamy R. The role of estradiol in male reproductive function. Asian J Androl. 2016;18(3):435–40.
37. Asadi N, et al. The impact of oxidative stress on testicular function and the role of antioxidants in improving it: a review. J Clin Diagn Res. 2017;**11**(5):Ie01–ie05.
38. Alahmar AT. Role of oxidative stress in male infertility: an updated review. J Hum Reprod Sci. 2019;12(1):4–18.