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EFFECTS OF NICOTINE EXPOSURE DURING GESTATION ON UTERUS AND OVARY OF FEMALE WISTAR RATS

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ABSTRACT

With more than 7000 chemical compounds, cigarette smoke; represents a major environmental risk to the health of the mother and her fetus. Nicotine is the main alkaloid of tobacco and is responsible for its addiction. The aim of our study is to assess the effects of nicotine on the uterus and the ovary of pregnant female rats. WISTAR rats with regular estrous cycle were divided into two equal groups; a control group and a group receiving 1mg/kg of nicotine daily from day 1 to day 19 of gestation. The rats were anaesthetized, their uteri and ovaries were removed, weighed and histologically examined. Serum estrogen and progesterone determinations were also performed. Our results showed that nicotine induced endometrial atrophy, follicular degeneration and a significant decrease in uterine weight and estrogen levels. Through these results, we suggest that gestational exposure of rats to nicotine affects steroidogenesis resulting in endometrial damage and follicular degeneration.

Key words: Nicotine, rats, ovary, uterus, histology.

INTRODUCTION

Cigarette smoke contains up to 7,000 chemical compounds including 69 carcinogenic agents(Noar et al. 2018).Several studies have shown that smoking cigarettes during pregnancy could raise the risk of prematurity, low birth weight, impaired child growth, and higher infant mortality (Liu et al. 2014; Pinheiro et al. 2015; Marufu et al. 2015). It is also associated with high risk of congenital malformations and functional disorders (Leppée et al. 2012). Additionally, women smokers face a greater risk of poor embryonic implantation and spontaneous loss of a pregnancy (Dechanet et al. 2011). Moreover, it has been reported in 2015 that 7.4% of the united states women were using e-cigarettes; 40% of them did not realize that ENDS contained nicotine and would be addictive (Scherman et al.2018).

Nicotine is one of the only chemicals consistently found in both forms of smoke (Tsai et al. 2020). It is the major alkaloid of tobacco. It is well thought out that nicotine is responsible for addiction to cigarettes. In fact, nicotine has deleterious effects on some vital visceral organs with observations similar to those reported in women smokers (Iranloye and Bolarinwa, 2009). Furthermore, the ovaries retain the compounds from cigarette smoke, creating a toxic environment that could disrupt follicular development and growth (Dechanet et al. 2011).

Nicotine affects human fertility in several ways including inhibition of aromatase enzyme activity in uterine granulosa cells, reduced urinary and plasma estradiol levels, and increased prevalence of early menopause with high rates of *in vitro* fertilization (IVF) failure (Sanders et al. 2002; Totonchi et al. 2016). Therfore, we suggested to study the effects of nicotine during gestation. The main purpose of

our study was to investigate if the physiological variations which occur during gestation would accentuate the reprotoxic effect of nicotine. the main objective of the present study is to evaluate the impact of gestational exposure to nicotine at a dose of 1mg/kg/day on the uterus and ovary, it is a multiparametric study including fertility index, relative organ weights, histological and hormonal analyses.

MATERIALS AND METHODS

Animals:

This study was performed on female WISTAR rats aged of two months and weighting 260±5 g, and with normal estrous cycle. Animals were purchased from Pasteur Institute in Algiers, Algeria. They were housed individually and exposed to a 12 h light/dark cycle with ad libitum access to food and water. All experiments were conducted according to the published guidelines of the care and use of laboratory animals in research.

Drug:

(-) NICOTINE PESTANAL, analtical standard (3050 Spruce Street , Sigma Aldrich, St. Louis, MO 63103, USA) was administered by gavage at 1 mg / kg / day(Cesta et al.2009).This dose was chosen to mimic the nicotine concentrations resulting from the consumption of 1-2 packs of cigarettes, used in previous studies conducted on laboratory rats(Holloway et al. 2006; Laule et al. 2017).

Study design:

Animals were divided into two groups; an experimental group that received nicotine from day 1 to day 19 of gestation and a control (Roguski et al. 2014) . Daily vaginal smears stained with 1% methylene blue were examined for at least two consecutive estrous cycles before the experiment to confirm that all the female rats used in the study had a regular oestrus cycle (Blake et al. 1972; Krinke, 2000).

Females were housed with males overnight (two females /one male). The next day, sperm-positive vaginal smears were used to confirm mating. Female rats were considered in day one of gestation (D1) if their vaginal smears contained sperm cells. On day 19 of gestation, females were anesthetized with chloroform (Mantella et al. 2013) and dissected. The fertility index was then calculated (number of pregnant females / number of coupled animals × 100) (Camargo et al. 2014). The ovary and uterus were removed, weighed and fixed immediately in 9% formalin for histological examination(Iranloye and Bolarinwa, 2009). Blood was collected by cardiac puncture. Samples were centrifuged for 15 minutes at 16 100 ×g and sera were separated and stored at -80°C until analysis (Halder et al. 2016).

Histological and serological analysis :

One uterus and one ovary from each animal were fixed in 9% formalin for 48 hours. Paraffin embedded tissue were then prepared and stained with hematoxylin and eosin, and the histo-morphology of the uterus and the ovaries was examined under light microscope (Halder et al. 2016). The identification of the different types of follicles was based on the classification of Pedersen and Peters. (1968).

Serum estrogen and progesterone determination was performed using COBAS e411 analyzer.

Statistical analysis:

Data were analyzed using SPSS version 26 software (IBM corporation and its licensors 1989, 2019) (Akpak et al. 2017). Organs weight, serum estrogen and progesterone levels from experimental and control groups were statistically analyzed using the student's t-test.

RESULTS

Fertility index:

The fertility index was 100% for both groups.

Effect of nicotine on genital organs relative weight:

Absolute ovarian weight of nicotine-intoxicated rats showed no significant difference compared to controls, whereas relative uterine weight was significantly decreased in rats of the nicotine-exposed group compared to controls ($P<0.05$) (Table 01).

Table 1: Ovary and uterus main weights of nicotine-exposed and control rats

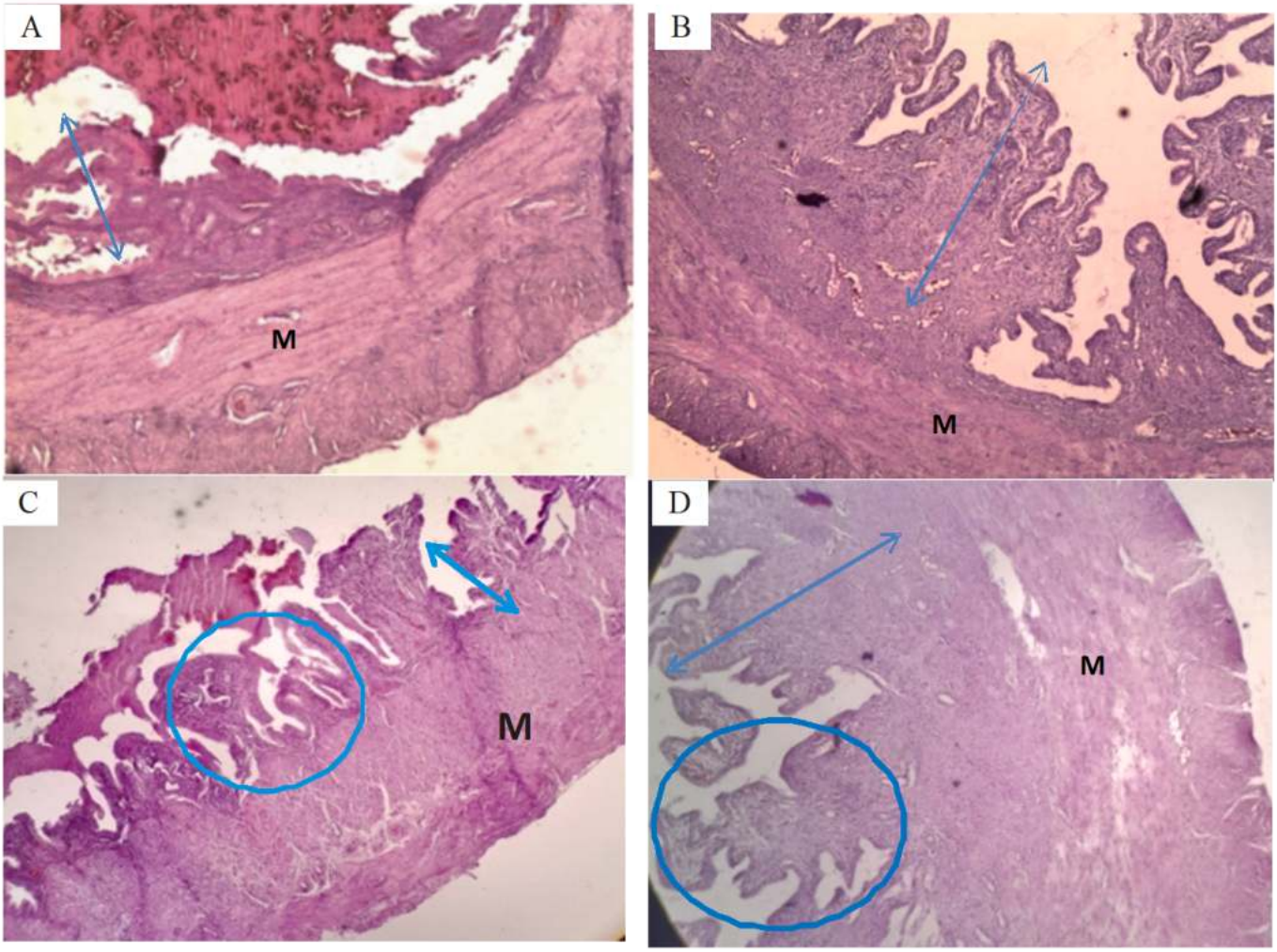
Group	Ovary weight (g)	Uterus weight (g)
Nicotine exposed group	0.124± 0.018	1.711± 0.089*
Controls	0.120± 0.010	1.930± 0.102

*A comparison between groups was made using the student test. Results are expressed as mean ± standard deviation; (n=5). * Significant difference ($p<0.05$), **very significant difference ($p<0.01$), ***highly significant difference ($p<0.001$).*

Effect of nicotine on uteri and ovaries histology :

Microscopic analysis of histological sections of the controls uteri showed that endometrial decidualization was uniform along the entire length of the uterine body (Figure1 b, d), whereas the degree of decidualization was considerably reduced and uterine villi were less developed in nicotine-exposed female rats (Figure1 a,c).

The myometrium development was reduced in nicotine-treated rats compared to controls (Figure 1 a, b, c , d), while vascularity (Figure 1 g , h) and uterine glands aspects were almost similar in the two groups (Figure 1 e , f).



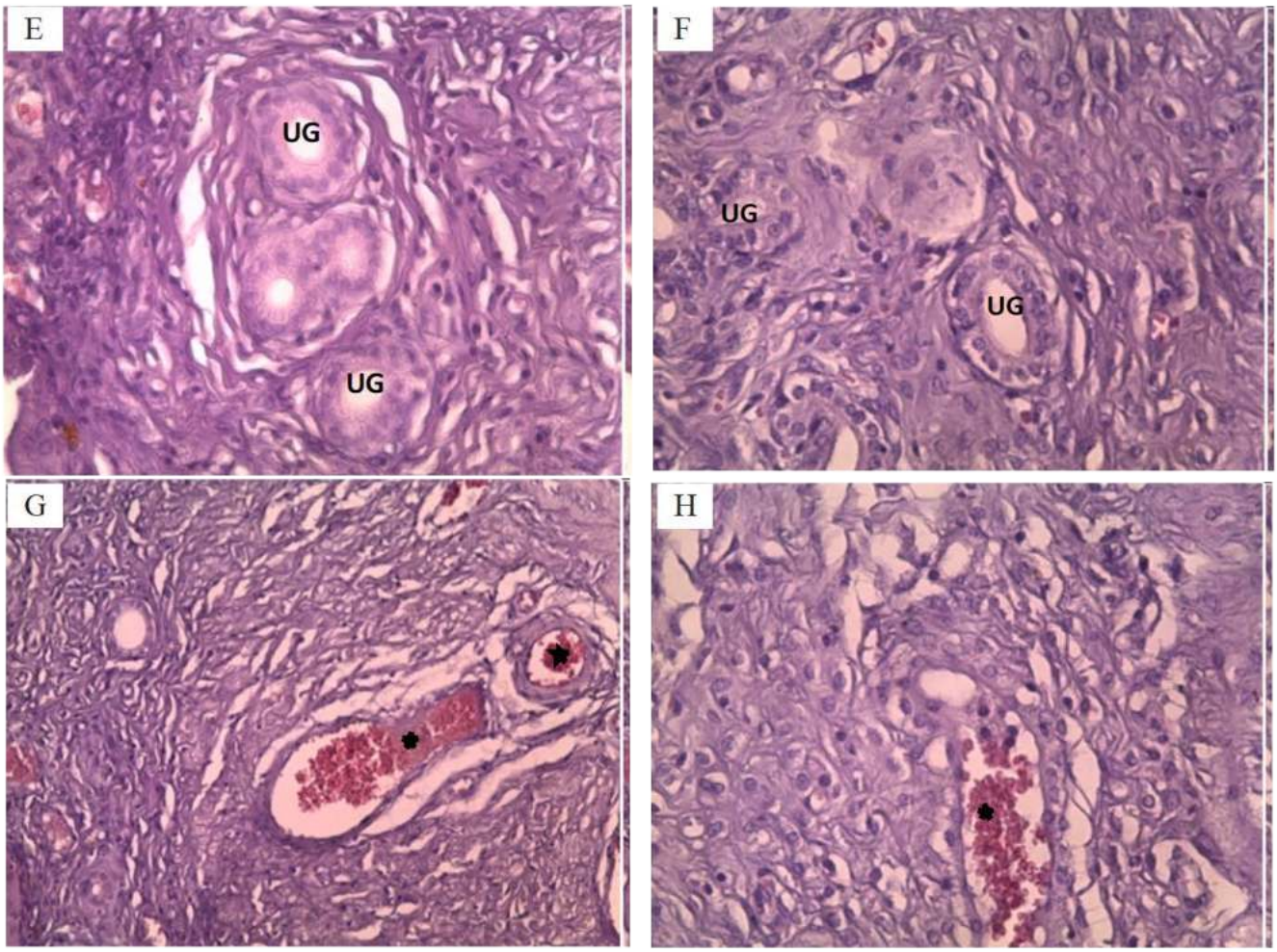
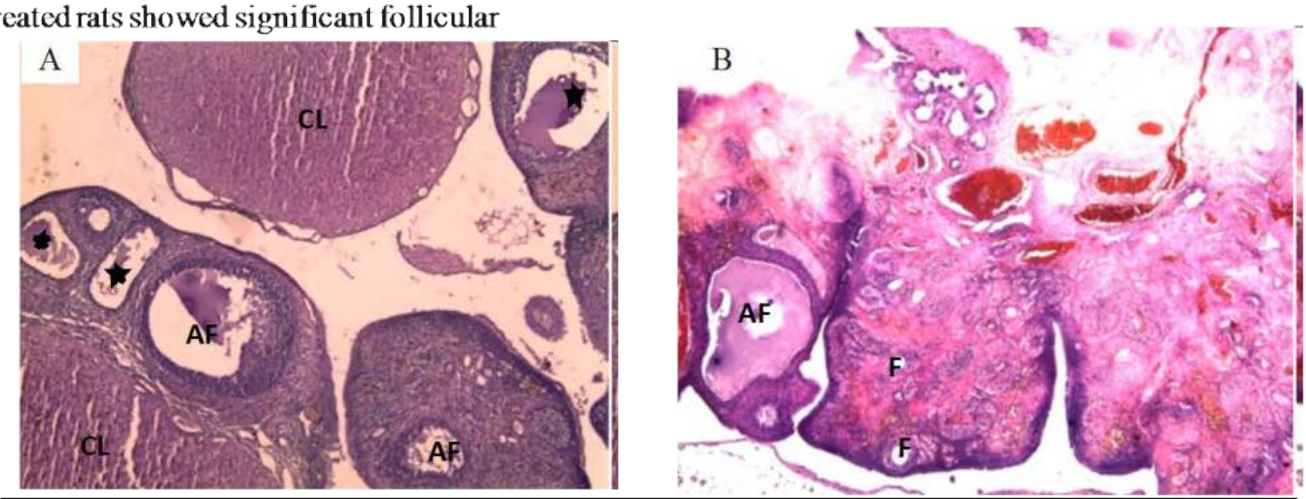


Figure 1 :Uteri histological changes induced by nicotine exposure during gestation in female rats

Endometrial decidualization in nicotine-exposed females (a) were reduced compared to controls (b) (G x10). Endometrium development was also reduced in nicotine-exposed rats (c) compared to controls (d), M: myometrium, (Gx40).Histological sections of the endometrium showed no changes in uterine glands (UG). Bewteen nicotine-exposed rats (e) and controls (f)., Vascularity was also unchanged in nicotine exposed rats (g) compared to controls (h);Star: blood vessels (Gx40).

Histological analysis of the ovaries from controls showed several follicles in different stages of development (Figure 2 b, d , f)) with numerous gestational corpora lutea(Figure 2, h). However, ovaries sections from the nicotine-treated rats showed significant follicular degeneration associated with fibrin deposition (Figure 2 e, g), the presence of numerous atresic follicles and cysts (Figure 2 a, c) and decreased number of gestational corpora lutea compared to controls.



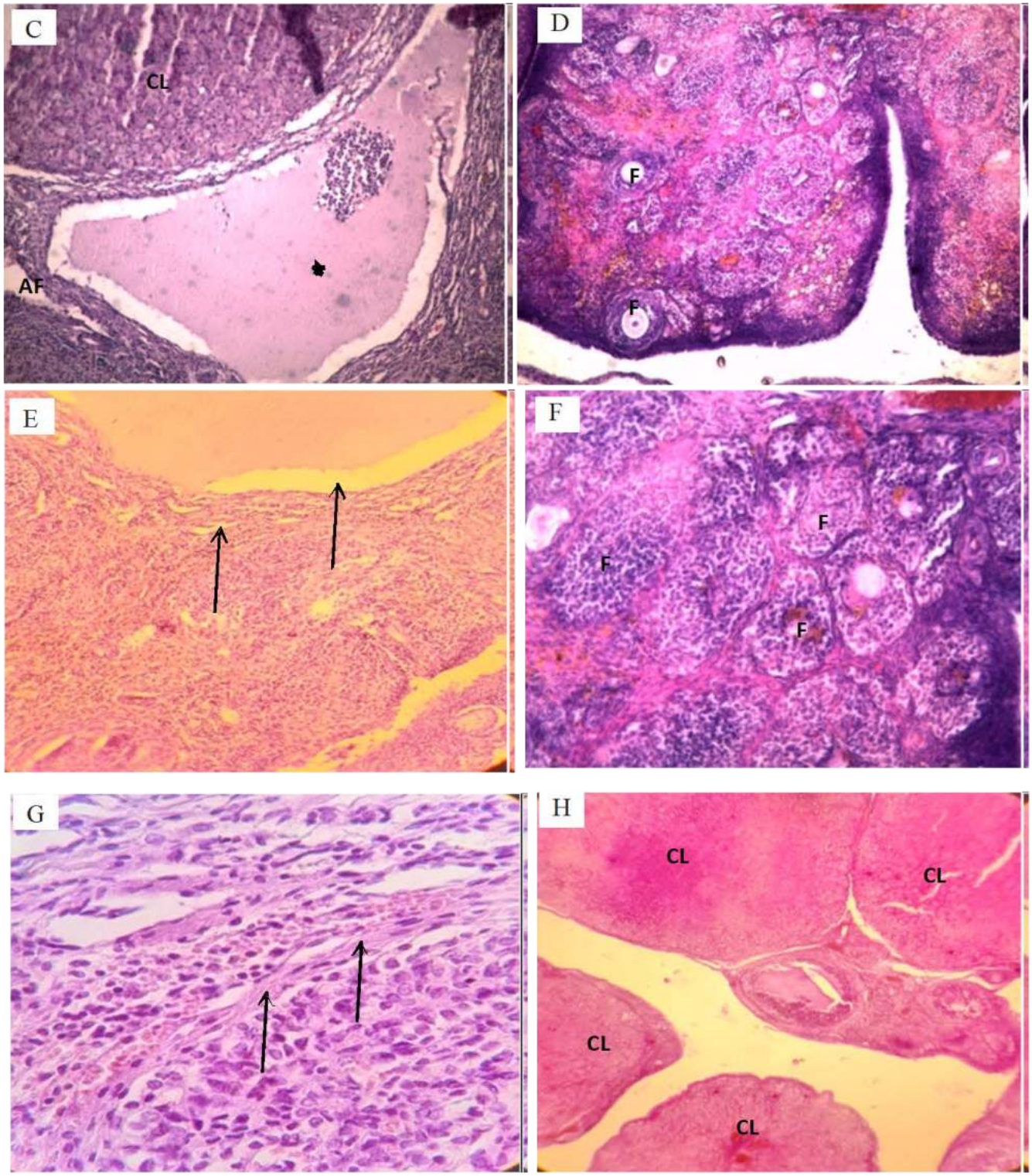


Figure 2: Effects of nicotine exposure during gestation on the ovarian histological structure in female rats H&E stain (G×40)

(a, c, e, g): strong presence of atresic follicles (AF), follicular cysts (Stars) and fibrin deposition (arrows) compared to controls (b, d, f, h) where we noted numerous developing follicles (F), more corpus luteum (CL) and less atresic follicles.

Sex hormone levels:

Serum progesterone levels showed no significant diffrencesbewteen nicotine-exposed group and controls, whereas estrogen levels were significantly decreased in nicotine-exposed rats ($p<0.05$) (Table 02).

Table 2: Serum progesterone and estrogen levels of nicotine-exposed rats and controls

Group	Progesterone in ng/ml	estrogen in pg/ml
Nicotine-exposed group	67.72 ± 8.80	22.84 ± 7.36*
controls	69.98 ±1.66	40.72 ± 1.30

*A comparison between groups was made using the student test. Results are expressed as mean ± standard deviation; (n=5). * Significant difference (p<0.05), **very significant difference (p<0.01), ***highly significant difference (p<0.001).*

DISCUSSION

We used an animal model to investigate the toxic effects of nicotine, the major addictive component of all forms of tobacco, on the structure and the functions of uterus and ovary during gestation.

In this study, gestational exposure to nicotine did not affect the relative weight of the rat ovary. This observation is supported by Siti Norashikin et al. (2014). However, it may depend on the duration of exposure. Longer nicotine administration periods of 30 to 60 days induced significant reduction in ovarian weight in exposed females (Iranloye and Bolarinwa, 2009).

On the other hand, the uterus appears to be more sensitive to nicotine exposure. In our study, we found that gestational exposure to nicotine significantly decreased the uterus relative weight in intoxicated rats compared to controls. Similarly, other researchers reported that gestational exposure during the first third of gestation affects uterine weight (Adeyemi et al. 2018). Nicotine induced the same effect in pseudo-pregnant (Siti Norashikin et al. 2014). The uterine weight decrease could result from a direct action of nicotine on this organ independently of the fetuses number. Iranloye and Bolarinwa. (2009) also linked the effects of nicotine on the uterine weight to the exposure duration. In his study, Bolarinwa noted that nicotine administration for 30 days did not reduce uterine weight, whereas after 60 days of nicotine treatment, uterine weight decreased significantly.

Nicotine negative effects did not spare the uterine endometrium. Our results showed that gestational exposure to nicotine negatively affects the uterine endometrium by reducing its decidualization. These histological changes were also observed in the uterus of female rats treated with nicotine during the first third of gestation (Adeyemi et al. 2018) , and in pseudo-gestant rats intoxicated by nicotine (Siti Norashikin et al. 2014). Although, other researchers have found no toxic effects of nicotine on the endometrial epithelium and stroma in female rats before and during gestation (Akpak et al. 2017). Development and maintenance of uterine endometrium decidualization are mainly controlled by ovarian estrogen and progesterone, which are regulated by hypothalamic and anterior pituitary hormones (Siti Norashikin et al. 2014). We suggest that nicotine affects the endometrium by disturbing the production of steroid hormones involved in the uterine endometrial decidualization process. Indeed, in nicotine-exposed rats supplemented with progesterone, decidualization was increased compared to the nicotine-treated group (Siti Norashikin et al. 2014).

In addition, our study showed that gestational exposure to nicotine increased follicular degeneration and atresia and the formation of follicular cyst in the ovaries of nicotine-exposed rats. Previous studies also reported that female rats intoxicated with nicotine for thirty days showed atrophied ovaries and intense follicular atresia associated with the formation of follicular cycts (Camargo et al. 2014). Other researchers have reported that exposure of female rats to nicotine for four weeks induced only follicular atresia and reduced the number of developing follicles (Roshankhah et al. 2017). Nicotinehas also been shown to significantly reduced the number of pre-antral and antral follicles and the concentration of

estradiol and increased the follicular atresia (Mohammadghasemi et al. 2012). Morover, it has been reported that nicotine inhibits the growth of ovarian follicles by inducing apoptosis of granulosa cells, which may result in negative effects on fertility (Bordel et al. 2006; Iranloye and Bolarinwa, 2009). In our study, although serum progesterone levels showed a slight non-significant decrease in nicotine-exposed rats, this decrease seems to be involved in the histological alterations mentioned above, taking into account the regenerative effect of progesterone treatment on decidualization (Siti Norashikin et al. 2014). Adeyemi et al. (2018) showed that the decrease in serum sexual hormones levels in nicotine-exposed rats during gestation was related to ovarian damage. We also found that serum estrogen levels were significantly decreased in nicotine-exposed rats compared to controls. The same results were found by Yang et al. (2014) and Adeyemi et al. (2018) who reported that gestational exposure of female rats to nicotine causes a significant decrease in serum estrogen levels. According to (Ruan and Mueck, 2015), nicotine affects steroidogenesis by decreasing aromatase activity in granulosa cells and peripheral tissues. Normal follicular development and the physiological changes of the endometrium during gestation are directly related to the action of estrogen. This implies that estrogens disturbance, whatever the cause, can be considered as the main reason of the uterine and ovarian histopathological alterations induced by nicotine during gestation.

Epidemiological evidence has shown that cigarette smoking is involved in several estrogen-related disorders in women including increased risk of early menopause (Baron et al. 1990). Animal studies revealed a toxic effect of cigarette smoke on the ovary, affecting follicular development and decreasing the sexual hormones (estrogens) levels (Marom-Haham and Shulman, 2016). Through our study, we can confirm that nicotine alone can also induce considerable negative effects on the reproductive organs involved in gestation. The oviarian and the uterine alterations were associated with a decrease in serum levels of sexual hormones, mainly estrogens, which could be attributed to granulosa cell apoptosis or aromatization defect. Those may also result from a direct cytotoxic action of nicotine on endocrine cells. Nicotine-induced hormonal disturbance may be one of the major mechanisms of nicotine toxicity on the ovary and uterus during gestation. Although, we were not able to determine other possible biochemical, biophysical and genotoxic mechanisms that may better explain nicotine toxicity during gestation.

CONCLUSION

Our study showed that gestational exposure to nicotine results in histological changes affecting the uterus and ovary; characterized by follicular atresia and uterine endometrial damage. Also, nicotine decreases ovarian reserve and interferes with steroid hormone synthesis. This results may contribute to a better understanding of the outcomes of gestational exposure to nicotine and provides information on possible mechanisms by which this exposure may contribute to reduced fertility in smokers pregnant women.

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Conflict of interest

We declare that we have no conflict of interest**.**

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