Effect of Cigarette Smoke on Spermatogenesis in Rats

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Introduction: The aim of this study was to evaluate the process of spermatogenesis in rats exposed to the cigarette smoke.

Materials and Methods: Thirty adult male rats were divided into 2 groups of cases and controls. An apparatus made especially for this study was used to produce smoke from a commonly used cigarette and expose the rats to the smoke. The rats in the case group were exposed to the cigarette smoke for 10 weeks (90 minutes every day for 6 days in each week). The rats in the control group were meanwhile in the fresh room air.

Results: Development of the sperms was mildly reduced in 14 (93.3%) and 4 (26.7%) rats in the case and control groups, respectively ($P < .001$). The mean average diameter of the seminiferous tubules was reported to be 0.421 ± 0.097 mm and 0.493 ± 0.026 mm in the case and control groups, respectively ($P = .04$). The mean numbers of Sertoli cells were 9.2 ± 1.2 and 13.3 ± 1.8 in the case and control groups, respectively ($P < .001$). A concurrent reduction in the number of germ cells and Leydig cells with the decrease in the number of Sertoli cells was seen in the rats of the case group.

Conclusion: Cigarette smoke has a rather obvious effect on spermatogenesis in rats which may be due to toxic substances in the cigarette or the histologic reactions due to hypoxemia induced by smoke. Although further documentation, especially in humans is required, the potential impact of smoking on fertility in men should be considered in public health education.

Keywords: smoking, spermatogenesis, animal model, rats, seminiferous tubules, Sertoli cells

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INTRODUCTION

Smoking and its complications are of the most important social and health problems in all countries.[1,2] Evaluation of the cigarette smoke on the urogenital system is very important especially in young population. Many studies have been performed on smoking and its deleterious effects on different parts of the body and reproductive system of animals and human.[3-6] In these researches, different methods of exposure to smoke, animal models, and period of exposure have been evaluated, the results of which show apoptosis in the progenitor cells of the testis, reduction in the number and epithelial height of the germ cells, problems in the function of the germ cell mitochondria, and increment in the oxygen-free radicals.[7-12] Due to the ethical matters and lack of access to testicular tissue, clinical trials cannot be performed on human. Thus, we evaluated the potential risks and complications of cigarette smoking on the process of spermatogenesis in an animal model. Owing to the similarity of the

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testicular tissues between the humans and rats, we used rats in this study.

MATERIALS AND METHODS

A total of 30 male rats of the Sprague race with the mean age of 10 weeks were purchased from the Khorasan Pastor Institute and were randomly assigned into 2 groups of cases and controls. The ethics committee of Mashhad University of Medical Sciences approved the study protocol. They were examined in the Experimental Research Center of Mashhad University of Medical Sciences. The cigarette we had chosen for this purpose was Pine Light with filter as a cigarette with average smoke production and price commonly used in Iran.

For achieving the smoke according to the predicted goals, a special apparatus was designed to have the ability to keep the rats for 2 hours in a very similar situation caused by smoking by human. Inspired based on the similar previous studies,[11-13] it had a VP800 vacuum suction with a 200-mL shield cylinder for condensation of the smoke, a glass box in a cube shape (aquarium shape) with the size of 30 × 40 × 80 cm for keeping the rats, and a hood over the aquarium-shaped box to evacuate the extra smoke from the environment (Figure 1).

Firstly, the rats in the case group were put in the box, and after closing the system, the cigarette was lit and the suction was concurrently turned on. The vacuum in the cylinder which was formed by the suction made the cigarette smoked. After finishing the cigarette, the suction was automatically turned off, and the smoke accumulated in the cylinder was moved to the aquarium by convection and exposed to the rats.

Each smoking procedure lasted 15 minutes including making the smoke and exposing the rats to the smoke for 10 minutes (1 minute, smoke condensation and 9 minutes, smoke exposure) and then, 5 minutes of rest and ventilation by uncovering the aquarium-shaped box and turning the hood on. This 15-minute operation was repeated 10 times a day for a total of 2.5 hours, yielding 90 minutes of exposure to smoke. Since the period of complete maturity of the sperms is about 52 days in rats, the time for smoke exposure was chosen to be 10 weeks. Each week, the rats were exposed to the smoke for 6 days, each day for 1.5 hours. Therefore, the rats in the case group were exposed to the smoke of a total number of 600 cigarettes.

During the study period, 15 rats in the control group were maintained in a similar place but exposed to the room air. After 10 weeks, the rats in the two groups were anesthetized by chloroform and then sacrificed by its slow increment in concentration. The testicular tissue was then excised, fixed in 10% formalin, and stained by hematoxylin-eosin. Two specimens were taken from each rat, each taken from one testicle. Histopathological examination was done by a single pathologist blinded to the rat groups. Development of spermatogenesis was classified into "normal," "mildly reduced," "severely reduced," and "no spermatogenesis" (Table 1). In addition, the average diameter of the seminiferous tubules in a microscopic field magnified at × 400, the mean number of Sertoli cells in 1 high-power field (HPF), and indexes of the Leydig cells and germ cells were determined. The indexes were calculated as follows:

Leydig cell index = Leydig cells per HPF/ Sertoli cells per HPF

Figure 1. The smoking apparatus is was constructed for simulating exposure of rats to cigarette smoke. **Left,** before exposure to smoke. **Right,** exposure to smoke.

Table 1. Pathologic Classification of Spermatogenesis

Class	Stages
Normal	Complete spermatogenesis and normal tubules
Mildly reduced	Normal sperm count but disorganized spermatogenesis Few sperms present
Severely reduced	No spermatozoa but abundant spermatids Few spermatids No spermatozoa or spermatids but abundant spermatocytes Few spermatocytes
No spermatogenesis	Only spermatogonia No germ cells but Sertoli cells No germ cells or Sertoli cells

Germ cell index = Germ cells per HPF/ Sertoli cells per HPF

Data analyses were performed by the SPSS software (Statistical Package for the Social Sciences, version 11.5, SPSS Inc, Chicago, Ill, USA). The chi-square test and the Mann-Whitney test were used for comparison of the status of spermatogenesis and the diameter of the seminiferous tubules, respectively. Evaluation of the mean Sertoli cells in a microscopic field was performed by the *t* test, since a normal distribution of the variable was noted. A *P* value of less than .05 was considered significant.

RESULTS

A significant difference was detected between the case and control groups regarding the effect of cigarette smoke on the process of spermatogenesis ($P < .001$). Development of the sperms was mildly reduced in 14 and 4 rats in the case and control groups (Table 2). Figures 2 and 3 demonstrate specimens with normal and mildly reduced spermatogenesis.

The mean average diameter of the seminiferous tubules was 0.421 ± 0.097 mm and 0.493 ± 0.026 mm in the case and control groups, respectively ($P = .04$; Figures 2 and 3). The mean numbers Sertoli cells were 9.2 ± 1.2 and 13.3 ± 1.8 in the case and control groups, respectively ($P < .001$). We did not found any differences between the two groups in the indexes of the Leydig cells and germ cells, due to the concurrent reduction in the number of germ cells and Leydig cells with the decrease in the number of Sertoli cells in the rats of the case group.

Table 2. Development of Spermatogenesis in Rat of Case and Control Groups*

Spermatogenesis	Case Group	Control Group
Normal	1 (6.7)	11 (73.3)
Mildly reduced	14 (93.3)	4 (26.7)
Severely reduced	0	0
No spermatogenesis	0	0

*Values in parenthesis are percents. The two groups were significantly different ($P < .001$).

DISCUSSION

Several studies have been performed on the harmful effects of smoking on the genital system of humans and rats.[1-16] Also, as it was previously mentioned,

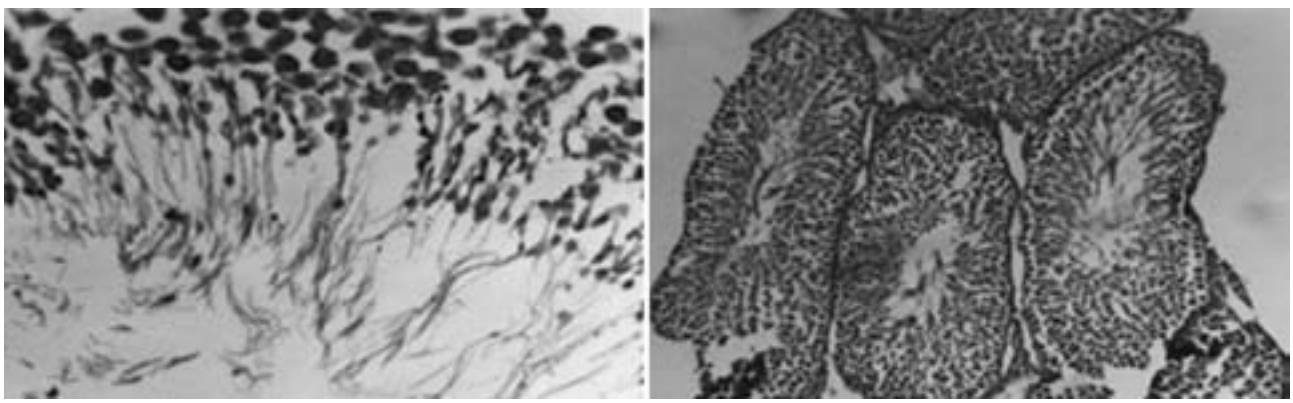

Figure 2. Testicular tissue of a rat in the control group. **Left,** the developing sperms. **Right,** the seminiferous tubules (hematoxylin-eosin, × 400).

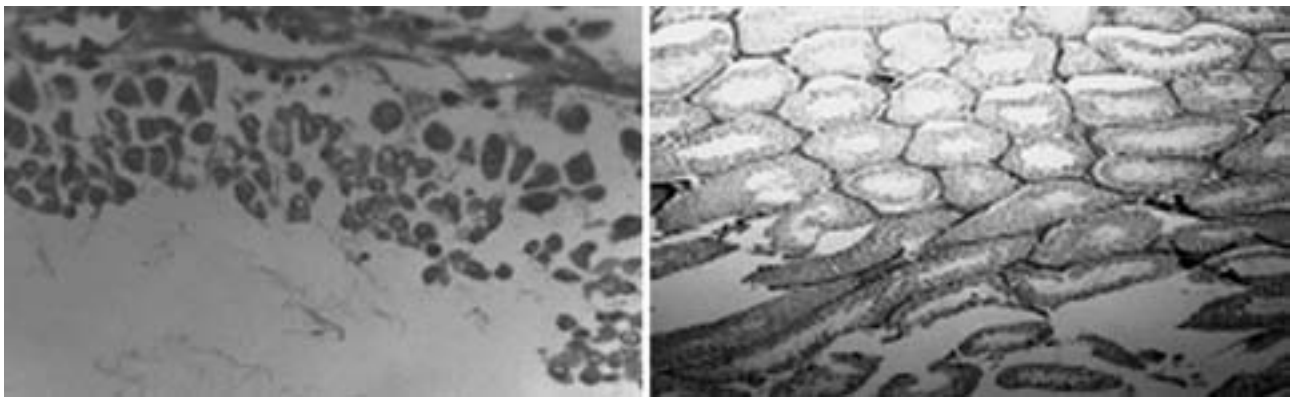

Figure 3. Testicular tissue of a rat in the case group. **Left,** the developing sperms with abnormalities in both number and maturity. **Right,** the reduced diameter of the seminiferous tubules and number of the Sertoli cells (hematoxylin-eosin, × 400).

the relationship between smoking and heart disease, lung disease, and cancers has been proved.[1,2,17,18] In 2 studies performed by Rajpurkar and colleagues, during the 15-, 30-, and 45-day periods, the effects of smoking were evaluated on the morphometric changes of the testicular tissue and showed reduction in the number of germ cells, decrease in the height of germinal epithelium, diameter of the tubules, and induced apoptosis in the genital cells of the testis.[11,12] Most of their results have been achieved in the present study as well, but we considered a 10-week study period to cover all spermatogenesis phases in rats (52 days), so that we were able to evaluate all possible changes during spermatogenesis that had not been evaluated previously. Of the factors that had not been well evaluated were the diameter of the seminiferous tubules, number and index of the Sertoli cells, percentage and quality of germ cells, and the Leydig cell and germ cell indexes.

In another study by Yamamoto and associates, reduced condensation and motility of the sperms, dysfunction of Leydig cells, and reduced ability of the genital system in hormonal secretions were shown after exposure of rats to cigarette smoke.[8] However, their results showed a few differences with ours that seemed to be due to the differences in methods such as the days of smoke exposure. The most important point in these all these studies is the apparent effect of cigarette smoke on the sperm development process in rats.

As we showed in this study, the process of spermatogenesis was impaired. In addition, the mean diameter of the seminiferous tubules and the index and number of the Sertoli cells reduced which could indirectly impair spermatogenesis. These changes may be because of the presence of many toxic substances in cigarette that affect all tissues including the testes. Also, the tissue reactions due to generalized hypoxia in the body can be another negative factor affecting the spermatogenesis in rats. However, the severity of smoke impact on the reproductive system is highly dependent on the using pattern, type, and number of cigarettes that are studied.[13-16] Due to the metabolic similarity of the human and rat tissues, it can be concluded that cigarette smoke may affect the sperm development process; however, this needs more research on human. Due to the importance of fertility in human and the high prevalence of smoking among general population, especially young people, smokers should be warned of the unwanted effects of cigarette smoking on fertility.

CONCLUSION

Our study showed a significant relationship between cigarette smoking and impaired testicular histology, reduced diameter of seminiferous tubules, and decrease in the index of the Sertoli cells in rats. All these elements are directly linked with the reduction in the sperm development process in rats which can be generalized to human; however, studies on human are warranted in this regard.

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CONFLICT OF INTEREST

None declared.

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