ORIGINAL ARTICLE

GMJ.2017;6(4):330-7
www.gmj.ir

Received 2017-05-29
Revised 2017-11-14
Accepted 2017-11-14

Assessment of the Protective Impact of Vitamin E on Sex Hormones and Sperm Parameters of Formaldehyde-treated Male Rats: A Preliminary Investigation

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Abstract

Background: Lifestyle factors, including environmental and occupational exposure, have a key role in reproductive health status and may impact fertility. Formaldehyde (FA) is a suspected reproductive toxicant, which may cause significant adverse effects on the reproductive system. This study was aimed at detecting the impact of FA and the possible protective role of vitamin E on the male reproductive system in rats. **Materials and Methods:** Thirty-two adult male Wistar rats were randomly divided into four groups: control rats, rats treated with vehicle (corn-oil), rats treated with 10 mg/kg/day FA (FAt), and rats treated with FA plus 30 mg/kg/day vitamin E plus vehicle (FAt+ vitamin E) for two weeks. After treatment, sex hormone levels were examined using ELISA. Moreover the count, morphology, and motility of sperm, were observed. **Results:** The sperm count and the percentage of rapid progressive sperm were significantly decreased in rats in the FAt-treated group compared with those in the control and vehicle-treated groups ($P<0.05$). Vitamin E treatment significantly improved the parameters examined in the FAt+ vitamin E group (29.85±8.62 vs. 10.04±4.79 for sperm count and 60.50±5.67 vs. 42.19±8.02 for sperm motility). Moreover, serum follicle-stimulating hormone (FSH) levels mildly decreased in the FA exposure group, although the difference was not statistically significant ($P>0.05$). **Conclusion:** The findings of this study revealed that FA exposure had a negative impact on sperm parameters and some reproductive hormones in rats and vitamin E attenuated the deleterious impact of FA on the reproductive system of adult male rats. **[GMJ.2017;6(4):330-7]**
DOI: 10.22086/gmj.v6i4.904

Keywords: Formaldehyde; Vitamin E; Rat; Reproductive Toxicity

GMJ

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Introduction

Infertility can present as a life crisis and cause psychological distress [1]. There have been serious concerns about the negative impact of environmental pollutants on human fertility [2]. Lifestyle factors including environmental and occupational exposure to toxicants have a key role in reproductive health status and may impact on fertility [3]. Among all the environmental pollutants, formaldehyde (FA) with more than 21 million pounds produced worldwide annually [4], is a major industrial chemical. It has been widely used for over 60 years in the production of resins, adhesives, and plastics [5,6]. It has also been used as a tissue preservative and disinfectant in anatomy and pathology laboratories, as an antimicrobial agent in cosmetics, as a fumigant in agriculture, and more recently in the production of crease-resistant garments and textiles [7]. Although most exposure to FA occurs in the work place, it can also occur in public areas, which makes it a threat to public health [5,8,9].Vitamin E is lipid-soluble, chain-breaking antioxidant, which protects biological membranes from oxidative damage [10]. Vitamin E has been reported to act as a free radical scavenger, preventing damage to the cell membrane by removing lipid peroxidation products and also by maintaining mitochondrial integrity and mediating generation of superoxide systems [11,12]. FA, a ubiquitous environmental contaminant, has long been considered a substance with harmful effects on the reproductive system. The protective effects of vitamin E on the brain and liver in rats exposed to FA has been described in previous studies [13,14], but the effects of vitamin E on FA-induced reproductive toxicity in rats has not been elucidated. Therefore, the present study was designed to investigate the adverse effects of FA on the reproductive system, and to determine whether vitamin E can reverse these effects. This study had two objectives. The first was to detect the impacts of FA on the reproductive system of male Wistar rats, and the second was to evaluate the possible protective role of vitamin E in alleviating the deleterious effect of FA on male fertility.

Materials and Methods

1. Animals

Adult male Wistar rats (250±20 g) were obtained from the Razi Vaccine & Serum Research Institute. They were fed a normal diet and tap water *ad libitum* and were housed in plastic cages under a 12-hour light/dark cycle and room temperature of 22–24ºC and 55–60% relative humidity. Rats adjusted to the environment for one week before being used in the laboratory. All procedures for utilizing rats were performed according to the standards of the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals [15] and approval of the Animal Ethics Committee of the Guilan University of Medical Sciences, Rasht, Iran.

2. Experimental Design

Thirty-two adult male Wistar rats were randomly divided into four groups by randomized block design (each comprising eight rats): control rats, rats treated with corn-oil as vehicle (VEH), rats treated with FA (FAt), and rats treated with FA plus vitamin E and corn oil (FAt+ vitamin E). FAt and FAt+ vitamin E groups were exposed to FA by intraperitoneal injection (IP) (10 mg/kg/day, Merck, Germany) for two weeks. Furthermore, rats in the FAt+ vitamin E group were administered vitamin E orally (30 mg/kg/day, dl-alpha-tocopherol, 21st Century Healthcare Inc., America) simultaneously [16].

3. Sample Collection

At the end of the 14-day exposure, rats were euthanatized under ketamine/xylazine anesthesia (ketamine 75 mg/kg + xylazine 15 mg/kg, IP, Sigma Co., Ltd, China). Blood samples were collected from the inferior vena cava of each animal. Blood was allowed to clot and then centrifuged (5000 rpm, 10 min) and the collected serum was stored at -70 °C until assays for the levels of reproductive hormones could be conducted [17]. In addition, the right cauda epididymis was finely minced into minute pieces and transferred into a prewarmed Petri dishes which

contained 5 mL T6 medium (Royan Institute, Iran) supplemented with 10% bovine serum albumin (BSA, Royan Institute, Iran) to allow sperm to swim out [18]. The minced tissue was placed in a 37°C incubator for 20 minutes.

4. Sperm Parameter Analysis

4.1. Sperm Motility

Sperm motility was evaluated immediately. The spermatozoa were assessed and classified as rapidly progressive (linear velocity ≥22 μm/s), non-progressive (linear velocity <22 μm/s and velocity ≥ 5 μm/s), and immotile spermatozoa. The number obtained in each category was expressed as a percentage and at least five microscopic fields were observed at 400× magnification under the microscope; at least 200 spermatozoa were observed for each sample. All methods were carried out at 37°C [19,20].

4.2. Sperm Count

The sperm count was performed using a Neubauer chamber (Improved Neubauer Hemocytometer, Deep 1/10 mm, LABART, Germany (under a light microscope at 400× magnification. The number of spermatozoa in five squares and on two sides of the Neubauer chamber was counted. The mean was multiplied by 10^6 to obtain the total number of sperm cells per mL of semen. The whole sperm (with heads and tails) were counted.

4.3. Sperm Morphology

Sperm morphology was evaluated by Papanicolaou stain method. An aliquot of stained sperm suspension was smeared on a glass slide and air-dried. A total of 100 spermatozoa from each animal were evaluated under a light microscope at 400× magnification and the total number of sperm with abnormalities was expressed as a percentage.All of the sperm evaluation steps were accomplished based on the World Health Organization manual for human sperm analysis with some modification [19]. To avoid inter-assay errors, all evaluation procedures were manually carried out by a single individual in duplicate for each animal.

5. Hormone Levels

Serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations were measured by an enzyme-linked immunosorbent assay (ELISA) using rat LH and FSH ELISA kits, respectively (Cusabio Co., Ltd., China) following the manufacturer's instructions. The sensitivity of the assay for LH and FSH was calculated respectively as 0.15 mIU/mL and 0.25 mIU/mL.Serum testosterone (TT) level was also measured using a total testosterone ELISA kit (Diagnostics Biochem Canada Inc., Canada) based on the manufacturer's protocol. The sensitivity of the assay was calculated as 0.22 ng. To calculate the hormone concentrations, a standard curve was plotted using standards provided in the kit. In order to avoid the effect of diurnal fluctuations, all samples were collected at set intervals in the early morning. Samples were measured in the same assay to minimize inter-assay variations.

6. Statistical Analysis

All statistical analysis was performed using SPSS version 18.0 software package (SPSS Inc. Chicago, IL, USA). Continuous variables were tested for normal distribution via the Shapiro-Wilk test and the results were expressed as mean±standard deviation (SD). The differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Scheffé's tests for post-hoc multiple comparisons. A p-value <0.05 was considered statistically significant.

Results

1.Epididymal Sperm Parameters

1.1.Sperm Count

The mean sperm count of rats in the FAt group was significantly decreased when compared with the control and VEH groups (P=0.003). Moreover, there was a significant increase in the mean value of the sperm count of rats that received 30 mg/kg vitamin E daily for two weeks when compared with rats that had received only FA (P=0.001, Table 1).

1.2.Sperm Motility

Significant reductions in the percentage of sperm with progressive motility were observed in rats of the FAt group when compared to the control and VEH groups (P<0.001). Vitamin E treatment significantly increased the percentage of rapid progressive sperm in rats of the FAt+ vitamin E group when compared to those of the FAt group (P<0.001, Table 1).

1.3.Sperm Morphology

The mean percentage of abnormal sperm morphology ranged from 29.71±2.13% to 34.42±4.72% in all treatment groups and no significant differences were found in the mean percentage of abnormal morphology among the examined, control, and VEH groups (Table 1).

2.Serum Hormone Levels

2.1.Follicle Stimulating Hormone

The results showed that treatment with FA caused a slight, albeit not significant, decrease in serum FSH levels when compared with the control and VEH groups. However, vitamin E treatment significantly increased FSH levels in the serum of FAt+ vitamin E treated rats when compared to the FAt group (P=0.042, Table 2).

Table 1. Sperm Count, the Percentage of Motile Sperm and Abnormal Sperm in Rats of the Study Groups.

Parameters / Groups	Sperm Count (10^6/ml)	Sperm Motility (Rapid Progressive, %)	Sperm Abnormal Morphology (%)
Control	23.75±4.76	67.19±5.56	29.71±2.13
Vehicle	27.28±9.45	66.00±5.19	34.00±4.04
FAt	10.04±4.79[a]	42.19±8.02[c]	34.42±4.72
FAt+ Vitamin E	29.85±8.62[b]	60.50±5.67[c]	34.33±3.83
p-value	<0.001	<0.001	0.085

FAt= Formaldehyde-treated
a= Significant vs. Vehicle group (P=0.003), **b=** Significant vs. FAt group. (P=0.001)
c= FAt vs. Control, Vehicle and FAt+ Vitamin E groups (P<0.001)

Table 2. Sex Hormones Level in Rats of the Study Groups

Parameters / Groups	LH (mIU/ml)	FSH (mIU/ml)	Testosterone (ng/ml)
Control	35.34±5.38	40.24±6.42	6.45±1.81
Vehicle	33.41±4.42	42.63±4.78	4.20±2.19
FAt	34.25±3.18	35.85±4.78	5.54±1.71
FAt+ Vitamin E	33.53±2.68	46.11±7.12[a]	6.14±1.10
p-value	0.780	0.035	0.089

FAt= Formaldehyde-treated
a= Significant vs. FAt group (P=0.042)

2.2.Luteinizing Hormone

No significant differences in serum LH levels were observed among the two treatment groups, or the control and VEH-treated groups (Table 2).

3.Testosterone

Similarly, the results showed that there were no significant differences in serum TT levels among the two treatment groups, or the control and VEH groups (Table 2).

Discussion

The present study showed there was a significant decrease in the sperm counts and motility with regard to the percentage of rapid progressive sperm in the FAt group (10 mg/kg, 14 days, IP). These results are in agreement with previous studies [16,21]. Moreover, no significant differences in sperm morphology, measured as the percentage of abnormal sperm, were observed among the groups. This finding is in accordance with Zhou and colleagues' report [16]. The mechanisms by which FA disrupts male spermatogenesis and fertility have been proposed by several studies. Zhou and colleagues reported that FA exposure significantly decreased levels of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione (GSH) activities and increased the levels of testicular lipid peroxidation products such as malondialdehyde (MDA) [16]. Our data is also consistent with results from other studies [22-24]. Thus, it is plausible to assume that oxidative damage and overproduction of reactive oxygen species (ROS) caused by FA exposure plays a decisive role in reproductive toxicity. ROS are products of normal cellular metabolism [25]. When ROS production exceeds the body's antioxidant defense mechanisms, it causes an oxidative stress response. The excess ROS induce peroxidative damage to cells and eventually leads to cell death [26].The sperm plasma membrane is rich in lipids in the form of polyunsaturated fatty acids, which may be easily attacked by ROS and are susceptible to peroxidative damage [17]. Sperm are highly sensitive to oxidative damage, which is mediated by lipid peroxidation leading to altered membrane function, impaired metabolism, morphology, and motility that eventually lead to infertility [26,27]. Disruption of the hypothalamus-pituitary-gonad (HPG) axis is another mechanism by which FA can disturb the male reproductive system. Sari *et al*. demonstrated that prolonged exposure to low levels of FA may lead to considerably altered responsiveness of the hypothalamus-hypophyseal-adrenal (HPA) axis such as an increase in corticotrophin releasing hormones (CRH)-immunoreactive (IR) neurons in the hypothalamus and adrenocorticotropin hormone (ACTH) cells in the pituitary [13]. Furthermore, Sorg *et al*. found changes in corticosterone hormone levels as a result of repeated low-level FA exposure [28]. Other mechanisms suggested for FA reproductive toxicity include epigenomic effects including DNA methylation, as well as apoptosis of germ cells and genotoxicity, however the exact mechanisms of action have yet to be understood [29]. In the present study, no significant associations were found between FA exposure and LH or TT serum levels. A small, but not significant, reduction in FSH serum levels was detected in the FAt group. This finding is consistent with Zhou *et al*. who observed that FA had no significant impact on LH, FSH, or TT levels [16]. Vitamin E is a major chain-breaking antioxidant that protects cell membranes from ROS and lipid peroxidation [10]. It has been recognized that vitamin E is a powerful antioxidant that is necessary for mammalian spermatogenesis [30]. Vitamin E has been reported to ameliorate adverse effects given its direct free radical scavenging properties, suppress lipid peroxidation in testicular microsomes, and restore the levels of GST and GSH to normal physiological levels [11,14,22]. The results of this study clearly show that daily oral administration of vitamin E for two weeks (30 mg/kg, 14 days) led to a significant increase in sperm count and the percentage of rapid progressive sperm, compared to rats exposed to FA but not treated with vitamin E. The protective effects of vitamin E on the total sperm count and sperm motility in some mammals have been reported previously [16,29-31]. However, no

significant improvements have been noted in the sperm morphology of rats treated with vitamin E. These data are in contrast to those reported by previous studies [16,31]. The dissimilarities may be due to the differences in the length, dose, or route of administration.
The published literature on the effects of vitamin E on hormone profiles of male rats exposed to FA is sparse. In the present study, a statistically significant increase in FSH serum levels was observed in vitamin E treated rats when compared with the FAt group values. Our study indicated that there was no considerable decline in serum TT or LH levels in vitamin E treated rats when compared with rats that received FA but were not treated with vitamin E.The limited amount of data based on a small sample size and relatively short period of exposure are the limitations of this study. A full cycle of spermatogenesis in rats requires 48–53 days [18]. Although a shorter exposure time may not be sufficient to affect spermatogenesis significantly, the duration of a single cycle of the seminiferous epithelium is about 8–10 days and our 14-day exposure time trial fulfilled that period [32]. Further investigation, with longer exposure and more subjects, is necessary to clarify the mechanisms of action of FA on the male reproductive system. Evaluation of appropriate oxidative stress biomarkers to characterize the effects caused by FA will also be helpful. Similarly, additional studies are essential to elucidate the precise role of vitamin E in ameliorating FA-induced reproductive toxicity.

Conclusion

The present study clearly identified that intraperitoneal administration of FA can induce a negative impact on sperm parameters and disturbances in hormone profiles in adult male rats. Furthermore, vitamin E provides appreciable protection against FA-induced reproductive toxicity probably though its free radical scavenging properties.

Acknowledgments

All hormone analyses were performed at the Cellular & Molecular Research Center Laboratory, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran. We are grateful to the staff of the Anatomy Department from the Guilan University of Medical Sciences for technical assistance and to Bahram Soltani, PhD, for providing advice on the ELISA protocol. We also thank Habibi MS for valuable remarks.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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