

## Aggravating effect of natural sand dust on male reproductive function in mice

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### Abstract

**Purpose** Although adverse health effects of environment (such as cadmium, pesticides, diesel exhaust, etc.) on the male reproductive system have been suggested, there is little experimental evidence of such an effect of atmospheric sand dust. In the present study, the effects of sand dust (mineral particles) were investigated on the male reproductive system of mice.

**Methods** Two types of sand dusts (Asian sand dust and Arizona sand dust) were intratracheally administered (0.1 mg/mouse 4 times every other week) to ICR male mice and then male reproductive organ weight, daily sperm

production (DSP), histological analysis and serum testosterone level were measured.

**Results** Histological examination showed that interstitial edema was produced by both sand dust types, and partial vacuolation of the seminiferous tubules was detected in the exposed mice. Moreover, exposure to these natural sand dusts significantly decreased DSP. On the other hand, there was no significant differences in serum testosterone concentration.

**Conclusions** These results suggest that natural sand dust-exposure produced adverse effects on mouse male reproductive function.

**Keywords** Daily sperm production · Intratracheal instillation · Male reproduction · Mineral particles · Sand dust

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### Introduction

Recent epidemiological studies showed a correlation between elevated levels of particulate matter (PM) and increased incidence of pulmonary infections, airway hyper-reactivity, and male reproductive function [1–5]. PM<sub>2.5</sub> (particle sizes less than 2.5 μm) and PM<sub>10</sub> (particle sizes of 2.5–10 μm) contain a variety of particles, such as industrial dust, diesel exhaust particles (DEP), sea salt, and sand dust (mineral particles) of soil origin. It has been estimated that up to 50% of the current atmospheric dust load originates from distributed surface soil [6]. Epidemiologic studies reported that sand dust events are associated with cardiovascular and respiratory problems, as well as an increase in daily mortality in Seoul, Korea [7, 8] and Taipei, Taiwan [9]. In recent experimental studies, data showed adverse effects of sand dust on murine lungs and aggravating

effects of sand dust on allergen-induced eosinophilic inflammation in murine airways [10–13]. Male fertility is inhibited by inflammatory disease [14–16]. However, there is a lack of data regarding the effects of sand dust on male reproductive function.

Airborne particles are composed of various metals, acid salts, organic pollutants, diesel exhaust particles and natural sand dust. Since diesel exhaust or carbon nanoparticles also aggravate antigen-related airway inflammation [17] and it was shown that diesel exhaust exposure or carbon nanoparticle treatment impairs male reproductive function in mice [18, 19]; it was postulated that sand dust may also affect this organ system. Because the effects of sand dust, one of the airborne particles, on the male reproductive system have not been previously investigated, the aim of present study was to examine effects of sand dust on male reproductive function in mice by histological observation of testicular tissue and daily sperm production (DSP).

## Materials and methods

### Experimental animals

A total of 48 male ICR mice (5 weeks of age, weighing 23–31 g) were purchased from CLEA Japan, Inc. (Tokyo, Japan) and quarantined for 1 week. Six-week-old mice (weighing 25–34 g) were then used in the experiment. Mice had ad libitum access to food (CE-2 commercial diet; CLEA Japan, Inc.) and water and housed in plastic cages lined with soft wood chips. The cages were placed in a conventional room, which was air conditioned to 23°C and 55–70% humidity with a 12-h light/dark cycle. The study adhered to the US National Institutes of Health guidelines for the use of experimental animals. The animal care method was approved by the Animal Care and Use Committee at Oita University of Nursing and Health Science in Oita, Japan.

### Materials

The present study used two types of natural sand dust, Asian sand dust (Asian SD) and Arizona sand dust (Arizona SD). Asian SD was previously collected from surface soils in the Shapatou Desert located on the southern fringe of the Tengger Desert, where dust storms occur frequently in north central China [13]. The Asian SD diameter of the samples (a total of 600 particles) was measured under a scanning electron microscope (JSM-5800, Jeol Ltd., Tokyo, Japan). The mean distribution peak of particle diameter in Asian SD was 6 µm. The Arizona SD was purchased from Powder Technology Inc. (Woonsocket,

RI). The mean distribution peak of particle diameter in Arizona SD was 6.6–8.6 µm.

Asian SD and Arizona SD were heated at 360°C for 30 min under 80% nitrogen gas in an electric heater to remove toxic materials (microbiological materials, sulfate, etc.) adhering to the particles. The amounts of LPS and β-glucan in original Asian SD (nonheated Asian SD) sample were consistent (3.66 EU/mg and 15.2 pg/ml, respectively). However, LPS and β-glucan were not detected in heated Asian SD, original Arizona SD, and heated Arizona SD samples. The contents of the mineral elements with oxide in Asian SD and Arizona SD were 60 and 68–78% for SiO<sub>2</sub>, 11 and 10–15% for Al<sub>2</sub>O<sub>3</sub>, 4.1 and 2–5% for Fe<sub>2</sub>O<sub>3</sub>, 1.8 and 2–4% for Na<sub>2</sub>O, 9.0 and 2–5% for CaO, 2.5 and 1–2% for MgO, 0.7 and 0.5–1% for TiO<sub>2</sub>, 2.2 and 2–5% for K<sub>2</sub>O, and 8.7 and 2–5% for less on ignition, respectively [12, 20].

### Study protocol

The ICR mice were divided into 3 groups ( $n = 16$  per group) according to exposure: control (administered vehicle alone), Asian SD and Arizona SD group. The particles were suspended in normal saline solution (Otsuka Co., Kyoto, Japan) for instillation. The instillation dose of Asian SD and Arizona SD was 0.1 mg per mouse according to a previously reported protocol [19]. A maximum deposition of approximately 30 µg into the lungs of a single mouse equals 100% deposition according to the Japanese national air quality standard for suspended particulate matter (0.1 mg/m<sup>3</sup>) to be accumulated in the lungs of one mouse per week (deposition efficiencies calculated by tidal volume, 0.15 ml per mouse, and breathing rate, 200 breaths per minute). The instillation dose (0.1 mg per mouse) of particles in the present study was 3.3 times that amount. However, the deposition rate into an alveoli, in the case of the human respiratory tract model for radiological protection, is approximately 3% at 5.5-µm particle diameter. The instillation dose of particles in the present study was 111 times the amount of approximately 0.9 µg in the case of 3% deposition. Asian dust storms with the Asian SD aerosol intermittently come flying over Japan during March and April (2 months) [20]. Therefore, this repeated instillation method was chosen to investigate the effects of natural sand dust on male reproductive function. Mice were intratracheally (i.t.) instilled with these particles under anesthesia with 4% halothane (Takeda Chemical, Osaka, Japan) using a polyethylene tube. The control mice were instilled i.t. with 0.1 ml of normal saline solution. The instillation of these particles or normal saline solution was repeated 4 times at 2-week intervals. At 12 weeks of age, and 24-h after the last administration, the mice were killed by exsanguination under anesthesia induced by

intraperitoneal injection of pentobarbital. Serum was prepared using CAPIJECT (Terumo Medical, NJ, USA) and frozen at  $-80^{\circ}\text{C}$  until assayed for testosterone.

Body and organ weights

Body weight was recorded and the weights of the testis and epididymis bilaterally were measured for each animal. After weighing, a piece of the left testis was fixed for histological observation and the right testis was frozen at  $-80^{\circ}\text{C}$  until thawed for sperm count analysis.

Light microscopy

Following exsanguination the testes of mice were fixed in Bouin’s solution, dehydrated with 70–100% ethanol, and embedded in paraffin. The sections were then stained with H&E and examined under a light microscope. To compare the extent of damage to the testes, the ratio of damaged seminiferous tubules, defined as those with degenerative and necrotic changes, desquamation of seminiferous epithelium to lumen, and loss of spermatozoon were observed, then calculated per cross section of each testis. Estimation of the testicular damage was conducted by counting the number of tubular cross sections and determining the percentage of total degenerated tubules in 3 cross sections per testis [19].

Daily sperm production per gram testis

A right testis sample was weighed and the seminiferous tubules released from the tunica albuginea into a saline solution in a homogenizer tube [21]. The testis was homogenized in 5 ml of lysis buffer using a Politron unit and left to stand for 60 min at room temperature. The mix was then diluted to the desired volume and the number of sperm heads or sperm cells counted in a hemocytometer chamber. The DSP/g was calculated according to the following formula:

$$\begin{aligned} &\text{Sperm number/ml} \times \text{volume of lysis buffer} = \text{total sperm number in testis} \\ &\text{Total sperm number in testis}/4.84^* = \text{sperm production/day} \\ &(\text{Sperm production/day})/\text{g testis weight} = \text{DSP/g (sperm/g/day)} \\ &^*4.84 \text{ is an invariable number for calculating sperm production per day in mice.} \end{aligned}$$

Assay for testosterone

Serum testosterone was measured in duplicate using an enzyme-linked immunosorbent assay (Testosterone ELISA

Test Kit, Endocrine Technologies Inc., CA, USA). Absorbance was measured at 450 nm by a microplate reader (SpectraFluor, TECAN, Grodig, Austria). The concentration of testosterone was calculated using standard curves. The detection limit of the assay was 20 pg/ml.

Statistical analysis

All results are expressed as mean  $\pm$  SD. Significant differences were examined by Dunnett’s pair-wise multiple comparison *t*-test using KyPlot Version 5 (KyensLab Inc., Tokyo, Japan). A *P*-value of  $< 0.05$  was considered to represent statistical significance.

Results

Effect of exposure to two sand dusts on body and organ weights and serum testosterone concentration

To determine the general toxicity of sand particles, body and reproductive organ weights were measured. The Asian SD group had a significantly lower mean body weight of 93.6% relative to control (Table 1). However, there were no significant differences in the organ weights between the control and treated groups. There were no significant differences in serum testosterone concentration between the control and treated groups.

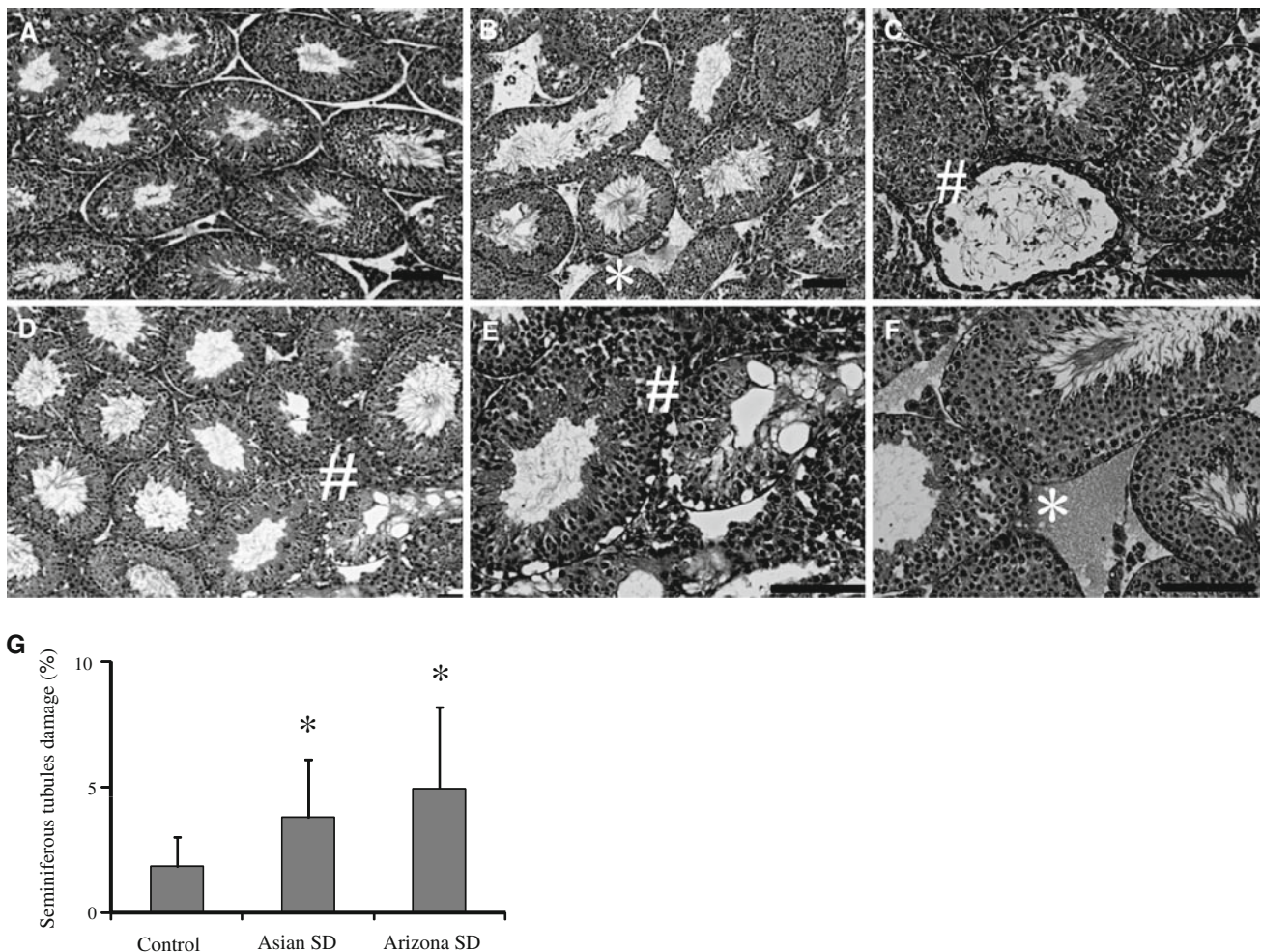
Morphological observation of testes in mice exposed to two sand dusts

In all testes of mice treated with the sand dusts, interstitial edema was observed by light microscopy (Fig. 1b, f). Moreover, vacuolation of the seminiferous tubules was observed in the both SD-exposed groups. The damaged tubules were scattered randomly throughout the testis (Fig. 1c–e). These effects were significantly higher in

**Table 1** Effect of natural sand dust on body weight and weight of reproductive organs and serum testosterone concentration

	Control	Asian SD	Arizona SD
Body weight (g) <sup>a</sup>	41.2 $\pm$ 2.6	38.6 $\pm$ 3.0*	43.5 $\pm$ 3.7
Testis (mg)	132.2 $\pm$ 17.4 <sup>b</sup> (3.2 $\pm$ 0.4) <sup>c</sup>	132.8 $\pm$ 19.1 (3.5 $\pm$ 0.5)	131.5 $\pm$ 20.2 (2.9 $\pm$ 0.9)
Epididymis (mg)	53.3 $\pm$ 4.6 <sup>b</sup> (1.2 $\pm$ 0.3) <sup>c</sup>	51.4 $\pm$ 4.8 (1.3 $\pm$ 0.1)	53.1 $\pm$ 6.7 (1.2 $\pm$ 0.3)
Testosterone (ng/ml)	4.4 $\pm$ 2.0	8.5 $\pm$ 7.6	11.2 $\pm$ 9.0

\* *P* < 0.05 compared with controls  
<sup>a</sup> Data are mean  $\pm$  SD values. *n* = 16  
<sup>b</sup> Absolute weight  
<sup>c</sup> Relative weight (mg/g body weight)



**Fig. 1** Morphological observations of the testes treated with Asian SD and Arizona SD mice. **a** Control; **b, c** Asian SD; **d–f** Arizona SD. Bar 100  $\mu$ m. Interstitial edema was observed in the testes in **b**, and **f** (asterisk). Some degenerated seminiferous tubules were observed in

the testes in **c–e** (hash). **g** The percentage of degenerated seminiferous tubules in cross sections of treated Asian SD and Arizona SD mice. Mean  $\pm$  SD ( $n = 16$ ); \* $P < 0.05$  compared with controls

Asian SD and Arizona SD-exposed groups than in controls (Fig. 1g). Although no remarkable morphological change was observed on Sertoli cells, Leydig cells and myoid cells of Asian SD or Arizona SD treated mice.

#### Daily sperm production

The DSP was significantly lower in the Asian SD and Arizona SD treated groups. The DSP decreased by 13% in the Asian SD group ( $P < 0.05$ ), by 18% in the Arizona SD group ( $P < 0.05$ ) compared to the control group.

#### Discussion

This study focused on the influence of natural sand dusts on the murine male reproductive system. There is extensive evidence that exposure to increased levels of inhalable

particulate pollutants ( $PM_{10}$  or  $PM_{2.5}$ ) is related to increased respiratory and cardiac morbidity, cancer and mortality [4, 22–24]. It was recently proposed that diesel exhaust (DE) may influence reproductive function [25, 26]. Exposure to 0.3, 1 or 3 mg diesel exhaust particles (DEP)/ $m^3$  for 6 months was shown to produce induction of Leydig cell degeneration, grater damaged seminiferous tubules, and reduction in daily sperm production (DSP) [18]. In our previous study, it was found that carbon nanoparticles (carbonaceous nuclei of DEP) administered at 0.1 mg/mouse at 10 one-week intervals produced induction of Leydig cell degeneration, a reduction in DSP and an increase in the testosterone concentration in serum [19]. However, there has been no study identifying the effects of natural sand dust in the environment on the male reproductive function of mice. The natural sand dust is inhalable particulate pollutants, same as carbon nanoparticles and DEP. Carbon nanoparticle and DEP are industrial products



or artifact, whereas the natural sand dust consists of natural material. It is important to determine the effects of PM<sub>10</sub> on the male reproductive function, because natural sand dust is one of the main components of PM<sub>10</sub>. On the other hand, human semen studies imply that PM<sub>10</sub> dust may exert a significant adverse affect on reproductive outcomes [27].

Our results indicate that two sand dust samples (Asian SD and Arizona SD) instilled i.t. markedly changed testicular morphology and decreased DSP. Thus, generalized natural sand dust (mineral particles) may aggravate the male reproductive system. Natural sand dust contains microbiological materials as well as chemical species such as polyaromatic hydrocarbons [28, 29]. In the present study, heat-treated sand dust samples were used to exclude the effects of these elements. On the other hand, it has been estimated that silica (Si) compounds comprised 60–80% of our two sand dust samples [12, 20]. Silicon dioxide (SiO<sub>2</sub>) reduced the sperm count and induced histological changes of testes in rat [30]. Elements, such as SiO<sub>2</sub>-containing SD, might well be related to the aggravating effects on the male reproductive system. Testicular histology demonstrated partial vacuolation of the seminiferous tubules in the Asian SD and Arizona SD groups. From an animal experimental point of view, several sand dusts considerably increase inflammation. Decreases of body weight in the SD treated mice may be associated with the inflammation [10]. Lipopolysaccharide (LPS) cause inflammation and aggravates spermatogenesis without affecting the serum testosterone levels in rats [14]. The present study also suggested that exposure to natural sand dust might induce changed testicular morphology, which may in turn have produced DSP reduction.

The present study suggests that natural sand dust exposure exerts adverse effects on mouse male reproductive function. For the present, it is not known whether sand dust poses a significant risk to the human male reproductive system. However, our data derived from the murine reproductive system suggests that various sand dusts may potentially influence spermatogenesis in men.

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