Original Article

The Effect of n-Hexane on the Gonad Toxicity of Female Mice*

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Abstract

Objective To investigate the toxic effects of n-hexane on the Ganod of female mice.

Methods n-Hexane was administered to four groups of mice by inhalation at doses of 0, 3.0, 15.1, and 75.8 mL/m^3 respectivelyfor five weeks. Each group consisted of 10 mice, of which half were injected in first with 10 IU of pregnant mare serum gonadotrophin (PMSG) on the 33rd days, and then with 10 IU of human chorionic gonadotrophin (HCG) 48 hrs later. After the treatment, mouse sera were sampled and ovulating hormone (LH), follicle-stimulating hormone (FSH), estradiol (E_2), and progesterone (P_4) levels were measured by electrochemiluminescence immunoassays (ECLIA). In each group, the right ovaries of the non-super-ovulated mice were stained with hematoxylin and eosin while ovaries on the left side were prepared with the TUNEL method in order to detect apoptotic cells.

Results The duration of the diestrus stage decreased significantly (P<0.05) in the 75.8 mL/m^3 group. All super-ovulated mice in each treatment group produced fewer eggs than those in the control group (P<0.05). The number of follicles in ovaries in the 75.8 mL/m^3 group was smaller compared with the control group (P<0.05).The serum P_4 levels in each treatment group were lower than those in the control group (F=6.196, P<0.01). The cell apoptotic rate in the 75.8 mL/m^3 group was higher (P<0.05).

Conclusion n-Hexane may have directly mediated via alterations hormone secretion and promoted granulosal cell apoptotic, which may be one of the important mechanisms for n-hexane induced mouse ovary impairment.

Key words: n-Hexane; Superovulation; Hormone; Ovary; Cell apoptotic rate

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INTRODUCTION

n-Hexane is widely used in industry as a solvent or a component of mixed solvents for production of vegetable oils, adhesives, paints, cleaning products, etc. Many cases of polyneuropathy due to n-hexane have been reported. Recently, studies have been found to indicate the toxicity of n-hexane for the female reproductive system. Some researchers conducted a retrospective study to examine the Time to Pregnancy (TTP) and found that exposure to n-hexane and other organic solvents was hazardous to female reproduction[1]. In Fujian Province, China, thirty workers in a shoemaking factory were poisoned by n-hexane, which damaged their reproductive systems and caused them to experience various symptoms such as decline in sexual function and menstrual abnormalities[2]. In addition, eleven workers in Hebei Province, China also experienced menstrual

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abnormalities after exposure to n-hexane[3].

There are thousands of shoemaking factories in which workers are often exposed to organic solvents such as n-hexane in China and the majority of workers in these factories are female. However, there is an astounding lack of studies on the gonad toxicity of this common chemical. This paper aims to help fill in the gap by studying the toxic mechanism of n-hexane and its effects on gonad based on the previous studies mentioned above.

The phenomenon of super-ovulation has proved to be a useful tool in the study of ovarian response to gonadotropic hormones. It is now evident that both the amount of ovulating hormone (LH) and the amount of follicle-stimulating hormone (FSH) play a major role in ovulation and super-ovulation. In this study, it is postulated that if n-hexane has an adverse effect on gonadotropin secretion, the number of oocytes would decrease after super-ovulation. Several experimental models on rats that represent specific and corresponding stages of follicular development have been established[4-7], in which apoptosis serves a precondition for normal oogenesis[8-11] and communication among neighboring granulosa cells, between granulosa cells and cumulus cells, and between granulosa cells and oocytes is essential for successful follicular growth and development.

This study attempts to explore the effects of n- hexane upon the gonad of mature female mice. Furthermore, it aims at finding out whether such effects are mediated via alterations in gonadotropin secretion or directly at the level of the ovaries.

MATERIALS AND METHODS

Animals

Forty female ICR mice of two months old (Shanghai Slac Laboratory Animal Co. Ltd, China) were used in these experiments. Each group contained 10 mice. They were habituated through regular handling for one week before the experiments. Ten mice per cage were housed in a room with controlled lighting (lights on 06:00-20:00), temperature (25±1 °C) and humidity (60%). The animals were fed with a mice diet and water ad libitum. The mice selected had at least three consecutive regular estrous cycles of four to five days in duration before initiation of the study.

Experimental Method

To study the effect of static n-hexane (Sigma Chemical Corp. St. Louis, MO, USA) inhalation on the gonad function of adult mice, mice were housed in an L-RDJ/1000 automatic static type exposure cabinet (Jiufang Electronics Corp. Guangzhou, China). n-Hexane was given to four groups of female mice for a five-week period (4 hours of exposure per day, 7days/week) at doses of 0,3.0,15.1, and 75.8 mL/m^3 respectively.

Estrous Cycle

At the last 2 weeks of n-hexane inhalation, the estrous cycles were investigated. Vaginal smears were made daily at fixed times (at 07:00 in the morning and at 19:00 in the evening, respectively) so that the stage of the estrous cycle could be determined[12-13]. We obtained the smears from each animal by rinsing the vagina with distilled water. After being dried, the smears were stained with hematoxylin for microscopic examination. Based on the observation of vaginal smears, the estrous cycle could be divided into four distinct stages, i.e., proestrus, estrus, metestrus, and diestrus. These stages were characterized in all groups by a moderate number of nucleated epithelial cells in proestrus, many cornified cells in estrus, leukocytes and cornified cells in metestrus, and nucleated epithelial cells and a larger number of leukocytes in diestrus.

Number of Ovulated Ova after Super-ovulation

On the 33rd day of n-hexane inhalation, super-ovulated mice in each group (five mice) were injected ip first with 10 IU of PMSG at 18:00 and then 10 IU of hCG diluted in PBS 48 h later (Sigma, St. Louis, MO). After twelve hours' exposure, the ovaries and oviducts were isolated. Ova were collected from the dilate ampullac of the oviducts for assessment of the final ovulation rate and counted under a dissecting microscope (Olympus, Japan). Ova were flushed out with M2 medium (Sigma, St. Louis, MO) containing 0.1 mg/mL hyaluronidase (Sigma, St. Louis, MO). The cell activity was observed under a dissecting microscope and the cell number was calculated with trypan blue (Sigma Chemical Corp. St. Louis, MO, USA) stain. Trypan blue staining is often used to identify the activity of eggs, and if eggs are dead, they will become blue.

Ovary Fixation and Follicle Counting

After exposure, the right ovaries of all non-super-ovulated mice in each group (five mice) were removed and incubated overnight in 4% paraformaldehyfive in PBS (pH 7.4), washed with 70%, 90%, 95%, and 100% ethanol and then embedded in paraffin. Tissue sections were cut from each mouse's ovary with rotary microtome (Yibiao

Corp, Shanghai, China). Thin sections (5μm) for follicle counting were consecutively stained with hematoxylin and eosin (H&E). Morphological identification and estimation of total primordial oocytes with a partial or complete layer of squamous granulosa cells[14], as well as primary (single layer of cuboidal granulosa cells), secondary (two or more layers of cuboidal granulosa cells but no visible antrum), natural (layers of cuboidal and visible antrum), antral (one or multiple visible fluid filled antral cavities), or corpus luteum (typically very large relative to the size of the ovary, just theca cells and granulosal cells) follicles were performed with a light microscope with a microcator to monitor section depth by using CAST-GRID software (Olympus, Albertslund, Denmark). Follicle nuclei (primordial) or nucleoli (primary and antral) were used as reference counting points. In serial sections, the first sections were selected randomly, and then all primordial and primary follicles were counted (×400 objective) to a depth of 18 μm in every fourth 5 μm section, and after that, whole ovary estimates were calculated by Cavalieri's principle with consideration of section (×4/1) and depth (×25/18) sampling. Section areas flanking the 18 μm counting depth allowed confirmation of follicle classification (e.g. primordial vs. primary) to avoid potential capping effects. Total secondary and antral follicle numbers were counted in every 5 μm serial section from each ovary (×400 objectives).

Hormone Measured

After exposure, blood was drawn (from the mice in each group, except the super-ovulated mice in each group), and the blood samples were placed at 4 °C for 3 h and were then centrifuged at 3000 rpm for 15 min. The supernatant was transferred to a 1.5-mL Eppendorf tube and stored at -20 °C . FSH, LH, E_2, and P_4 were measured by using Elecsys according to the manufacturer's instructions (Elecsys analyzer Roche Diagnostics). All the samples were measured at the same time to minimize errors.

Transmission Electron Microscopy

Some of the right ovaries in each group of non-super-ovulated mice were removed. The samples were fixed in 3% (vol/vol) glutaraldehyde in 0.05 mmol/L cacodylate buffer (pH 7.2) overnight and postfixed with 1% osmium tetroxide in the same buffer for 1.5 hour at 4 °C , typically very large relative to the size of the ovary. They were then dehydrated in an ascending series of ethanol solutions and embedded in epoxy resin[15]. En face sections were cut from embedded samples and stained with lead citrate and uranyl acetate and examined under a transmission electron microscope (model HU-12A, Hitachi Crop, Japan).

Determination of Apoptotic Cells by TUNEL

In order to detect apoptotic granulosal cells in mice ovaries, the left ovaries and ovarian sections of the non-super- ovulated mice in each group (five mice) were stained by the terminal deoxynucleotidyl transferase-mediated biotiny- lated deoxyuridine triphosphate nick end- labeling (TUNEL) method by using a commercial kit (Sigma, St. Louis, MO) according to the manufacturer's instructions. The sections were dehydrated in a graded ethanol series, cleaned in xylene at room temperature and immersed in 0.3% H_2O_2 in methanol for 10 min to inhibit endogenous peroxidase activity. They were then incubated in Tunel solution for 1 hour at 37 °C . After being incubated with peroxidase-labeled anti-DIG antibody solution for 30 min at room temperature, the sections were incubated with BICP/NBT solution for 20 min at room temperature, mounted with Entellan (Merck) and examined by a light microscope.

Statistical Analysis

Data were expressed as means±SEM, and were subjected to one-way ANOVA followed by a Dunnett test or a Student-Newman-Keuls multiple comparison test. SigmaStat statistical software (SPSS) was employed. Significant differences were established at $P \leqslant 0.05$.

RESULTS

General Appearance

In the experiments, mice in each group all appeared quiet to different degrees. In the 75.8 mL/m^3 group, the mice exhibited decreased activity, depilation, decreased appetite, as well as rhabdomyolysis and ulcers in the abdominal area. Yet, only one animal in the 75.8 mL/m^3 group died during the experiments.

Mice Body Weight

The mice body weights were different after exposure to n-hexane. The body weights in the 75.8 mL/m^3 group decreased as time went by. Compared to the control group, the mice's weights in the 75.8 mL/m^3 group decreased (P<0.05) (Figure 1).

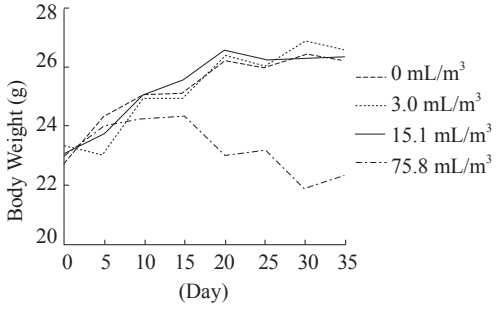


Figure 1. Mice body weights after exposure to n-hexane ($\bar{x}$±s, g).

Estrous Cycle

The change in the estrous cycle is shown in Table 1. The results show that the estrous cycle stage was abnormal when mice exposed to n-hexane compared to the control group. The duration of the diestrus stage decreased significantly in the 75.8 mL/m^3 group (12.00±0.000) compared to the control (22.50±7.690) (P<0.05). In general, mice with exposure to n-hexane exhibited an abnormal estrous cycle.

Table 1. The Stage of Estrus Cycle after Exposure to n-Hexane (Mean±SEM, hour,▲*means P*<0.05)

Group (mL/m^3)	*n*	Proestrus	Estrus	Diestrus	Metestrus
0	8	24.00±12.829	60.00±30.086	22.50±7.690	56.00±29.857
3.0	8	20.50±8.401	66.86±39.104	15.38±5.423	62.00±32.338
15.1	8	44.63±6.428	72.50±24.883	18.88±8.741	56.00±41.898
75.8	8	30.00±23.127	55.50±25.607	12.00±0.000▲	34.25±14.945

Number of Ovulated Ova after Super-ovulation

The effects of different concentrations of n-hexane on the number of ovulated ova after super-ovulation are shown in Figure 2 and Figure 3. The results of the experiments (Figure 2) show that compared to the control group (46.50±13.69) the number of ovulated ova in 3, 15.1, and 75.8 mL/m^3 groups after exposure to n-hexane decreased significantly (28.50±4.71, 28.50±2.55, and 22.50±8.28 respectively) (P<0.01). The death rate of ovulated ova increased significantly (P<0.01, P<0.05) in the 15.1 and 75.8 mL/m^3 groups (0.0499±0.0272 and 0.0663±0.0333) compared to the control (0.0165±0.0263) (Figure 3).

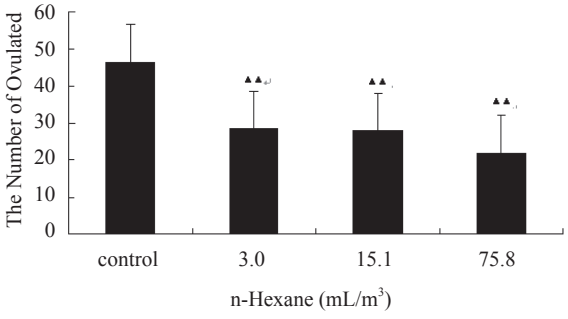


Figure 2. We counted the number of ovulated ova after super-ovulation in each group, in which we scarified 5 mice and counted the ovulated ova under the light microscope. ▲▲*means P*<0.01.

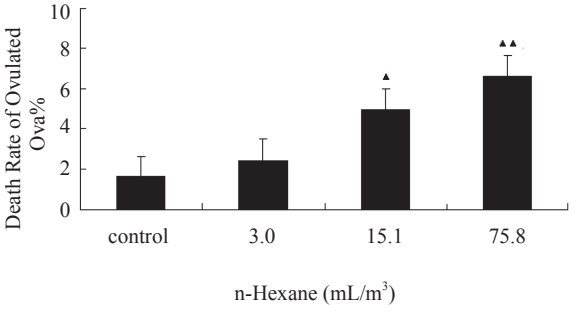


Figure 3. Death rate= the death of ovulated ova/total ovulated ova in all the mice in each group. ▲*means P*<0.05, ▲▲*means P*<0.01.

Follicle Numbers

The effects of different concentrations of n-hexane on the ovaries as determined by histopatho-logical observations are shown in Table 2. The results show that the composition ratio of the number of follicles of all types in the high-dose n-hexane group decreased (P<0.05, P<0.01), while the mature follicle ratio in the ovaries decreased (P<0.05) in the 15.1 mL/m^3 group compared to the control.

Table 2. The Effect of n-Hexane on the Number of Mice Follicle (%)

Group (mL/m^3)	Primordial Follicle	Pri/sec Follicle	Mature Follicle	Atresic Follicle	Corpus Luteum/albicans
0	17.81	54.80	10.27	3.43	13.70
3.0	14.63	56.10	13.82	3.25	12.20
15.1	18.69	56.91	7.31$^{▲}$	4.07	13.00
75.8	21.57$^{▲}$	46.08$^{▲▲}$	5.01$^{▲▲}$	8.81$^{▲▲}$	19.61$^{▲▲}$

Note. Chi-square test; Compared with the control group. $^{▲}$*means P*<0.05, $^{▲▲}$*means P*<0.01.

Hormone Measured

The change in the level of hormones in the serum is shown in Table 3. The results indicate that there was no significant difference in FSH, LH, and E_2 serum levels compared to control animals (*P*>0.05).

However, the results (Figure 4) also reveal that the P_4 serum levels decreased significantly (*P*<0.01) in the 3.0, 15.1, and 75.8 mL/m^3 groups (3.5722±2.2212, 3.5029±2.0238, and 2.4500±0.6277 respectively) compared to the control (6.8800±3.4585).

Table 3. The Effect of n-Hexane on the Serum Hormones FSH, LH, and E_2 Levels

Group (mL/m^3)	FSH (mIU/mL)	LH (mIU/mL)	E2 (pmol/L) ($\bar{x}$±s)
0	1.6744±0.3443	0.9875±0.3683	46.0750±20.2417
3.0	1.8510±0.3346	1.0725±0.5633	39.2170±23.0842
15.1	1.8438±0.4731	0.9163±0.4534	38.7670±19.6961
75.8	1.7954±0.3747	1.2171±0.6341	37.9640±15.2556

Note. One-way ANOVA; Compared with the control group.

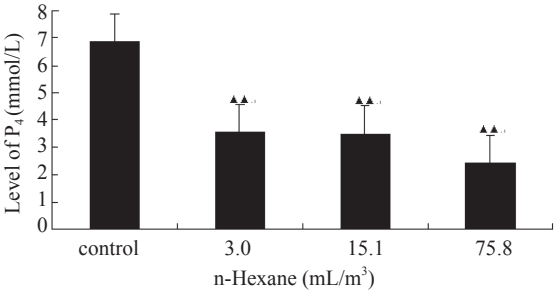


Figure 4. We matured the level of P_4 in all of the mice in each group (Mean±SEM). *F*=6.196, $^{▲▲}$*means P*<0.01.

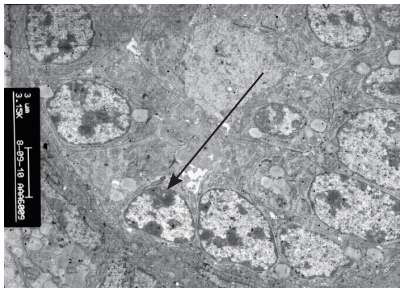
Electron Microscopy

In the experiments, single and aggregated cells taken from the control group displayed well-developed mitochondria and numerous round osmiophilic lipid-containing secretory droplets, characteristic of steroidogenic cells. The matrix of the nuclei appeared physiological, with normal chromatin condensation (Figure 5). However, many of the cells taken from the high-exposure group showed a dramatic increase in chromatin condensation in the nucleus (Figure 5). Furthermore, all of the groups exposed to n-hexane exhibited uniformly dispersed nucleoli within the nucleoplasm, where one or two large nucleoli were also found (Figure 5). The cytoplasm taken from the 75.8 mL/m^3 group contained damaged mitochondria with ruptured internal membranes, damaged lipid droplets, and autophagic vesicles (Figure 5).

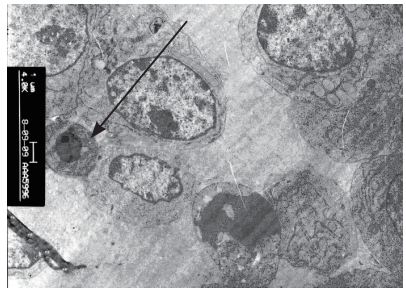
Determination of Apoptotic Cells by TUNEL

The effects of the different concentrations of n-hexane on granulosal cell apoptotic in the ovaries are shown in Table 4 and Figure 6. Apoptotic granulosal cells ratio=the apoptotic granulosal cells in different stage of follicle / the granulosal cells in whole follicle in one slice, and we counted 35 slices. The results show that the granulosal cells' apoptotic rates in mature follicles were significantly different in the 15.1 and 75.8 mL/m^3 groups compared to the control (x^2=7.82, *P*<0.01; x^2=11.31, *P*<0.01, *P*<0.05,

P<0.01). The apoptotic rates in atresic follicles show significant difference in the 15.1 mL/m^3 group compared to the control (x^2=7.36, P<0.01). Also in corpus luteum, the apoptotic rates of the 15.1 and 75.8 mL/m^3 groups were significantly different from those the control (x^2=4.28, P<0.05; x^2=8.33, P<0.01).



Control group (× 3150)



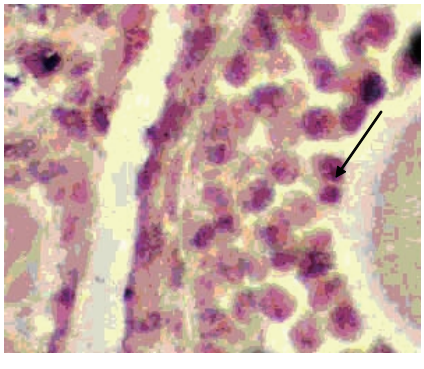
75.8 mL/m^3 group (× 4000)

Figure 5. The plasma and intracellular membranes of ovaries are labeled. The arrow in the left picture shows the normal granulosal cell in the control group while the arrow in the right picture indicates the apoptotic granulosal cell.

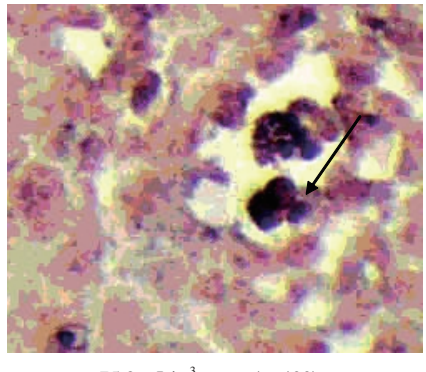
Table 4. The Effect of n-Hexane on the Apoptotic Granulosal Cells (%)

Group (mL/m^3)	Primorial	Pri/sec	Mature	Atresic	Corpus Luteum
0	0.49	2.21	3.83	17.33	25.25
3.0	0.65	2.64	3.56	18.15	30.81
15.1	0.53	1.58	7.68▲▲	30.11▲▲	33.19▲
75.8	0.78	2.47	10.57▲▲	20.62	43.34▲▲

Note. Chi-square test; Compared with the control group ▲*means* P<0.05; ▲▲*means* P<0.01.



Control group (× 400)



75.8 mL/m^3 group (× 400)

Figure 6. Photomicrography of apoptosis on the ovaries after exposure to n-hexane (×400). The arrow in the left picture shows the normal granulosal cell in the control group, while the arrow in the right picture indicates the apoptotic granulosal cell.

DISCUSSION

Over the past decade, there has been increasing concern about the health hazards of human exposure to n-hexane. It has already been established that in several species, n-hexane induces deleterious effects on the eyes[16], nerves, and liver as well as the reproductive organs[1]. So far, however, there have been very few studies investigating the effects of n-hexane on various reproductive processes in the female, especially when it comes to exposure to different concentrations of n-hexane during different stages of the estrous cycle. Most studies have focused on peripheral neuropathy[17]. In our previous study, we found that the main active metabolite of n-hexane 2,5-hexanedione (2,5-HD) caused increased

apoptosis in human ovarian granulosa cells[18]. We undertook this study in hopes of finding out the effects of n-hexane and the mechanism of its gonad disruption so as to make it clear its mediation role in hypothalamic- pituitary-ovary axis. It is hoped that findings from this study can be used to help shape public health policies benefiting thousands of women whose ability to have children is now being potentially endangered by their jobs in factories.

In order to determine whether the effects of n-hexane on gonad disruption were mediated with the hypothalamic-pituitary-ovary axis, we performed three different experiments including estrous cycle measurement, super-ovulation phenomenon and follicle development. In addition, the serum hormone of mice was also measured to explore to what degree the n-hexane's gonad disruption mechanism could mediate the hypothalamic-pituitary-ovary axis . The findings were as followed:

1. Changes in the frequency of the estrous cycles can indicate whether there is a gonad disruption. In this study, two consecutive estrous cycles were measured to determine whether fluctuations in these parameters might occur in response to a changing exogenous environment. There have been reports of workers in shoemaking factories who have menstrual abnormalities apparently resulting from exposure to n-hexane[3]. It could be found from this study that the duration of the estrous cycle stage decreased incrementally with increased exposure to n-hexane. The diestrus stage decreased significantly (P<0.05) in the 75.8 ml/m^3 group compared to the control. This result suggests that exposure to different concentrations of n-hexane can cause menstrual abnormalities in mice.

2. Ovarian response to gonadotropic hormones is another index used to estimate a chemical's gonad toxicity. It could be learnt from a previous study on super-ovulation that ovaries of mice exposed to cadmium were not affected by super gonadotropic hormones[19]. In the present study, we found that the number of ovulated ova decreased in each group (P<0.01). Also the death rate of ovulated ova increased significantly (P<0.05, P<0.01) in the 15.1 and 75.8 mL/m^3 groups compared to the control. Our results show that n-hexane exposure inhibits the response of the ovaries to super gonadotropic hormones.

3. Follicles are the main functional units within the ovaries. There are follicles of several stages in the ovaries including primordial follicles (oocytes with partial or complete layer of squamous granulosa cells), primary follicles (oocytes with single layer of cuboidal granulosa cells), secondary follicles (oocytes with two or more layers of cuboidal granulosa cells but no visible antrum), mature follicles (oocytes with three or more layers of cuboidal granulosa cells and visible antrum), and antral follicles (oocytes with one or multiple visible fluid filled antral cavities)[14]. Many follicles are recruited to develop during the estrous cycle, but only a few are selected to ovulate. The follicles that are not selected to ovulate undergo atresia. Exogenous factors that have gonadal toxicity could accelerate or delay follicular development. Some papers have reported that n-hexane-treated ovaries exhibit follicular developmental disorder. Experiments in the present study show that the number of follicles at all stages decreased in the high-dose n-hexane group (P<0.05, P<0.01). Under normal circumstances, the total number of follicles in the ovaries is fundamentally constant. Our results suggest that exposure to n-hexane inhibited follicular development and promoted follicular atresia and luteal degeneration. It is known that disruption of the hypothalamic-pituitary-gonadal axis by endo- genous and/or exogenous factors, leading to aberrant secretion of LH, causes abnormal ovarian follicular development. Our findings suggest that n-hexane may have an effect on gonad disruption mediated indirectly via alterations in gonadotropin secretion. Studies have also shown that serum hormone was an important factor in the regulation and development of follicles. Serum estradiol and progesterone concentrations are closely related to follicular development. Estradiol has been shown to inhibit apoptosis of follicular granulosa cells and promote follicular development[20]. Damage to the estradiol hormone could therefore result in insufficient follicular development and increased apoptosis similar to what we observed in groups exposed to n-hexane.

4. In our study, we found that after the inhalation with different doses of n-hexane, serum progesterone (P_4) levels decreased significantly, which was statistically significant compared to the control group (P<0.01); however, serum estradiol (E_2), FSH and LH levels did not change. This indicates that n-hexane has an apparent gonad interference function, especially on progesterone levels. Nevertheless, the individual differences in serum were huge. In the study, we just used 4-5 mice to test the serum and then found that serum estradiol (E_2), FSH and LH levels did not change. We could also not rule out the possibility that the samples were not enough, which led to no change in serum estradiol (E_2), FSH and LH levels.Therefore, further researches

are also needed.

It could be summed up from the findings mentioned above that n-hexane inhibited the serum progesterone levels and had direct harm on the ovary, which may serve a reason for n-hexane's impact on mice estrous cycles and follicular development. No effect of n-Hexane has been found on the pituitary gland, and this may be due to the functional compensation of pituitary gland which has kept FSH and LH at normal levels. Further investigation from this aspect is needed.

In recent years, studies have found out that apoptosis is the potential mechanism of ovarian follicular atresia, and that environmental factors are important mechanisms of ovarian injury[21]. In our study, electron microscopy results indicate that, as the exposure dose of n-hexane increases, ovarian cell ultrastructure sustains increasingly serious damage. Brown granules appear in the nucleoli of oocytes, granulosa cells and mesenchymal cells, and as their chromatin becomes pyknotic, cell volume decreases, cytoplasmic organelles gets compact, and cell density increases. A qualitative analysis of the electron microscopy results in which all the aforementioned changes were observed indicates that n-hexane may lead to apoptosis in ovarian cells.

In addition, TUNEL study results show significant differences in the apoptotic rates of mature follicles, atresic follicles and corpus Luteum ($P<0.05$, $P<0.01$) in comparison with the control group. These results suggest that follicular dysplasia in the experiments may be due to ovarian cell apoptosis.

Our study shows that n-hexane has obvious female sexual gonadal toxicity. In the high-dose group, the length of the diestrus stage of the estrous cycle decreased significantly, the number of abnormal estrous cycles observed in each group increased significantly and the members of the high-dose group exhibited follicular development disorder at all levels of follicular development. After exposure to n-hexane, the response of the ovaries (super-ovulation) to excessive levels of gonadotropins significantly decreased. Unfortunately, the response of n-hexane exposed ovaries to normal levels of gonadotropins remains unknown. n-Hexane can inhibit progesterone a level, while n-hexane can lead to apoptosis of granulosl cells, both of them appears to be one of the important mechanisms leading to ovarian damage.

REFERENCES

1. Sallmen M, Neto M, Mayan ON. Reduced fertility among shoe manufacturing workers. Occup Environ Med, 2008; 65, 518-24.
2. Wang Wenyong, Wu Ansheng, Lin Xing et al. Investi gated n-hexane poised in Quanzhou and Putian City. Strait Journal of Preventive Medicine, 2003; 01, 31-2.
3. Xu Xuechun. Chronic n-hexane poising in clinic. Industrial Disease, 2004; 30 (3), 175-7.
4. Dhanasekaran N, Moudgal NR. Bio- chemical and histological validation of a model to study follicular atresia in rats. Endocrinol Exp, 1989; 23, 155-66.
5. Shi F, LaPolt PS. Relationship between FoxO1 protein levels and follicular development, atresia, and luteinization in the rat ovary. J Endocrinol, 2003; 179, 195-203.
6. Bigelow KL, Fortune JE. Characteristics of prolonged dominant versus control follicles: follicle cell numbers, steroidogenic capabilities, and messenger ribonucleic acid for steroidogenic enzymes. Biol Reprod, 1998; 58, 1241-9.
7. Calder MD, Manikkam M, Salfen BE, et al. Dominant bovine ovarian follicular cysts express increased levels of messenger RNAs for luteinizing hormone receptor and 3 beta-hydroxysteroid dehydrogenase delta(4), delta(5) isomerase compared to normal dominant follicles. Biol Reprod, 2001; 65, 471-6.
8. Manabe N, Inoue N, Miyano T, et al. Ovarian follicle selection in mammalian ovaries: regulatory mechanisms of granulosa cell apoptosis during follicular atresia. In: Leung PK, Adashi E (eds.), the Ovary 2nd edition. Amsterdam: Academic Press/ Elsevier Science Publishers, 2003, 369-85.
9. Manabe N, Goto Y, Matsuda-Minehata F, et al. Regulation mechanism of selective atresia in porcine follicles: regulation of granulosa cell apoptosis during atresia. J Reprod Dev, 2004; 50, 493-514.
10. Matsuda-Minehata F, Inoue N, Goto Y, et al. The regulation of ovarian granulose cell death by pro- and anti-apoptotic molecules. J Reprod Dev, 2006; 52, 695-705.
11. Matsuda-Minehata F, Maeda A, Cheng Y, et al. Regulation of granulosa cell apoptosis by death ligand- receptor signaling. Anim Sci J, 2008; 79, 1-10.
12. Koto M, Miwa M, Tsuji K, et al. Change in the electrical impedance caused by cornification of the epithelial cell layer of the vaginal mucosa in the rat. Jikken Dobutsu, 1987; 36, 151-6.
13. Montes GS, Luque EH. Effects of ovarian /steroids on vaginal smears in the rat. Acta Anat, 1988; 133, 192-9.
14. Meredith S, Dudenhoeffer G, Jackson K. Classification of small type B/C follicles as primordial follicles in mature rats. Journal of Reproduction and Fertility, 2000; 119, 43-8.
15. Ibaraki N, Lin LR, Reddy VN. Effect of growth factors on proliferation and differen tiation in human lens epithelial cells in early subculture. Invest Ophthalmol Vis Sci, 1995; 36, 2304-12.
16. Valerio Carelli, Flavia Franceschini, Silvia Venturi. Grand Rounds: Could Occupational Exposure to n-Hexane and Other Solvents Precipitate Visual Failure in Leber Hereditary Optic Neuropathy? Environmental Health Per spectives , 2007; 115(1), 113-5.
17. Sendur OF, Turan Y, Bal S, et al. Toxic neuropathy due to N-hexane: report of three cases. Inhal Toxicol, 2009; 21(3), 210-4.
18. Yan Sun, Yuan Lin, Hong Li, et al. 2,5-Hexanedione induces human ovarian granulosa cell apoptosis through BCL-2, BAX, and CASPASE-3 signaling pathways. Arch Toxicol, 2011. Spe; 8.
19. Zhang Wenchang, Wu Zhiren, Li Huangyuan, et al. Effects of Cadmium as a possible Endocrine Disrupor upon the Serum level of Sex Steriods and the Secretion of Gonadotropins from Pituitary in Adult Rats. Acta Med Nagasaki, 2002; 47, 53-6.
20. Menchaca A, Pinczak A, Rubianes E. Follicular recruitment and ovulatory response to FSH treatment initiated on day 0 or day 3 postovulation in goats. Theriogenology, 2002; 58(9), 1713-21.
21. Vaux DL. Apoptosis timeline. Cell Death Differ, 2002; 9, 349-54.