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Effects of Cadmium on Female Fertility Parameters: An Experimental Study

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ABSTRACT The aim of the present study was to evaluate the effects of cadmium administration on female infertility parameters in mice. A total of 54 female mice were randomly allocated into three study and three control groups. Cadmium (200 ppm) was added into the water of study groups for 30, 60 and 90 days. The histopathological examination of vaginal smears of mice as well as evaluation of serum levels of estradiol, luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin was performed. Administration of cadmium for 30 days caused minor histopathological alterations in the morphology of vaginal smears. 60-day and 90-day exposures of cadmium resulted in thinning of vaginal epithelium and increase in apoptotic cells. Prolonged exposure to cadmium was associated with significantly decreased serum levels of estradiol. However, levels of luteinizing hormone, follicle-stimulating hormone and prolactin did not differ between control and study groups. The results have shown that cadmium may contribute to unexplained female infertility.

INTRODUCTION

Infertility is defined as the inability to become pregnant during one year despite regular sexual intercourse in the absence of any contraceptive methods (Weiss 2014). Changing socioeconomic conditions, delay of the age of marriage, popularization of contraception methods and increasing prevalence of sexually transmitted diseases lead to an increased incidence of infertility. The prevalence of infertility in couples of reproductive age ranges between ten and fifteen percent (Weiss 2014).

Recently, various environmental factors such as smoking, alcohol, heat, electromagnetic waves, radiation, estrogen and chemical substances have been supposed to play a role in infertility etiopathogenesis (Fowles 2012). Heavy metals such as cadmium, lead, aluminum, chromium and strontium are taken into the body via the oral route. Exposure to these heavy metals is linked with adverse effects on human fertility (Sengupta 2015; Rzymski 2015). Cadmium is a heavy metal that is usually found together with zinc in nature. The most important source of cadmium exposure is supposed to be cigarette smoking. The incidence of smoking in women of reproductive age is twenty-five percent, and thirteen percent of female infertility has been linked with smoking (Waylen 2010).

This experimental study was based on an evaluation of histopathological alterations in vaginal smear and changes in serum levels of estradiol, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin. Cadmium was administered independently from phases of the estrous cycle in mice. Another important aspect of this study is to raise awareness of nursing researchers on experimental research.

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Objective

Contemporary scientific trends necessitate popularization of experimental research in nursing as well as other medical disciplines. The aim of the present study was to evaluate the effects of cadmium on fertility parameters in mice.

METHODOLOGY

This experimental study was performed in the experimental research laboratory of a university between October 2013 and February 2014. Before the study, the approval of local research ethics committee for animal experimentation was obtained (2013.05.02-05).

A total of 54 adult female Balb/c mice weighing 20-30 g were used. Animals were allocated into groups of 3-4 in each cage and kept under standard conditions of temperature (22-25°C), humidity (60%), and in a silent and well-aerated room. Day and night cycles of 12 hours were administered, and *ad libitum* access to water and food (21% raw protein) was allowed.

Control groups consisted of 8 mice, while there were ten mice in each study group. To minimize variability of estrous cycle and fertility, mice were randomly assigned into study and control groups.

In control groups named 1, 2 and 3 (n=8) mice were fed on *ad libitum* access to water and food supply. Intracardiac blood samples were obtained on 30^{th}, 60^{th}, and 90^{th} days respectively. In study groups 1, 2 and 3 (n=9, n=9, and n=7 respectively), cadmium chloride (200 ppm) was added into the water for 30, 60 and 90 days and intracardiac blood samples were obtained at 30^{th}, 60^{th} and 90^{th} days, respectively.

Vaginal smears were taken to determine the estrous cycle and observe the histopathological alterations in various groups. The three months control of the regular course of the estrous cycle before the experiment was performed to identify the ovulation periods. Levels of FSH, LH, estradiol and prolactin were studied to assess the impact of cadmium on fertility parameters.

Vaginal Smears

Estrous phases were followed by taking vaginal smears every morning at 09:00. To determine the transition from estrous phase to metestrous phase, another vaginal smear was obtained at 20:00 on the same day from subjects detected to be at the estrous phase in the morning smear.

Vulvar regions of mice were cleansed with seventy percent ethyl alcohol and distal parts of vulva were lubricated using cotton swabs. After this preparation, smears were obtained gently from the vagina and spread on a glass slide. After drying the slides at room temperature, seventy percent methanol was applied on slides for fixation. Toluidine blue of one percent concentration was administered (*Sigma-Aldrich, St. Louis, MO, USA*) for 5 minutes and washed with distilled water. Subsequent to coverage with Canadian balsam (*Sigma-Aldrich, St. Louis, MO, USA*), views of ovulatory phases were received using CX31 Olympus light microscope and C-5060 Olympus camera (*Olympus Corporation, Shinjuku, Tokyo, Japan*). The sections were examined in the Histology and Embryology Department of the institution.

Blood Samples

All mice were anesthetized by an intraperitoneal route using 50 mg/kg Ketamine HCl (10 ml) (*Sigma-Aldrich, St. Louis, MO, USA*) and 5 mg/kg Xylazine HCl (50 ml) (*Sigma-Aldrich, St. Louis, MO, USA)*. Mice were stabilized in a supine position, and 5-10 ml of intracardiac blood samples were drawn at ovulation periods. Serum samples were separated by centrifugation at 3000 rpm at 4°C for 10 minutes. Beckman Coulter Analyzer LH 780 device (*Beckman Coulter, Brea, CA, USA*) was used to analyze these blood samples with original kits.

Statistical Analysis

The data derived from this study has been analyzed using Statistical Package for Social Science (SPSS) 16.0 version program (*SPSS Inc., Chicago, IL, USA*). Normal distribution of serum estradiol levels was tested with the Kolmogorov-Smirnov method. Descriptive data was expressed as mean ± standard deviation or median (minimum-maximum).

Every study group was compared to its corresponding control group (for example, study group 1 versus control group 1, study group 2 versus control group 2, and study group 3 versus control group 3), and the Bonferroni-corrected Mann-Whitney U test was used for this purpose. A statistical method for this comparison was the Kruskal-Wallis test, and level of significance was set at $p<0.05$.

RESULTS

During the experimental study period, six mice from the control groups and three mice from the study groups died. Therefore, six mice remained in each of three control groups while the final numbers of mice in study groups 1, 2 and 3 were 9, 9 and 7, respectively.

Histopathological Results

Before Cadmium Exposure

Isolated mice have an estrous cycle of 4-5 days. This cycle is characterized by proestrous, estrous, metestrous and diestrous phases, and

cellular profiles detected in vaginal smears have typical features in conjunction with these phases.

Smears obtained from subjects during three months before the onset of the experiment revealed a regular course of proestrous, estrous, metestrous and diestrous phases. In the proestrous phase, the typical nucleated epithelial cells forming clusters were remarkable. Mucinous cells with nuclei at an apical location were noted in advanced stages of proestrous phase (Fig. 1a). Smears indicating a ninety percent predominance of cornified cells without nuclei were accepted to be in estrous phase. If smears acquired at 20:00 were consistent with enlarged cells constituting big clusters, these findings were assumed to be indicative of ovulation and mice were sacrificed at this stage. In metestrous phase, dense collections of nucleated epithelial cells and neutrophilic infiltration can be observed (Fig.1b). Diestrous phase was characterized by a more apparent neutrophilic infiltration accompanied by a relative diminution of epithelial cells (Fig. 1c). Thin vaginal epithelium is typical for diestrous phase.

After Cadmium Exposure

Study group 1 that had been exposed to cadmium chloride (200 ppm) for 30 days revealed a more prominent cluster formation by nucleated epithelial cells. Other phases did not yield any additional distinctive features. In Figure 2, views of proestrous (2a), estrous (2b), metestrous (2c) and diestrous (2d) phases after a 30-day exposure to cadmium are shown.

Cellular characteristics observed in vaginal smears of study group 2 after an exposure to cadmium for 60 days are seen in Figures 3a-d. Apoptotic cells have remarkably increased in number compared to study group 1. In Figure 3, views of proestrous (3a), estrous (3b), metestrous (3c) and diestrous (3d) phases after a 60-day exposure to cadmium are shown.

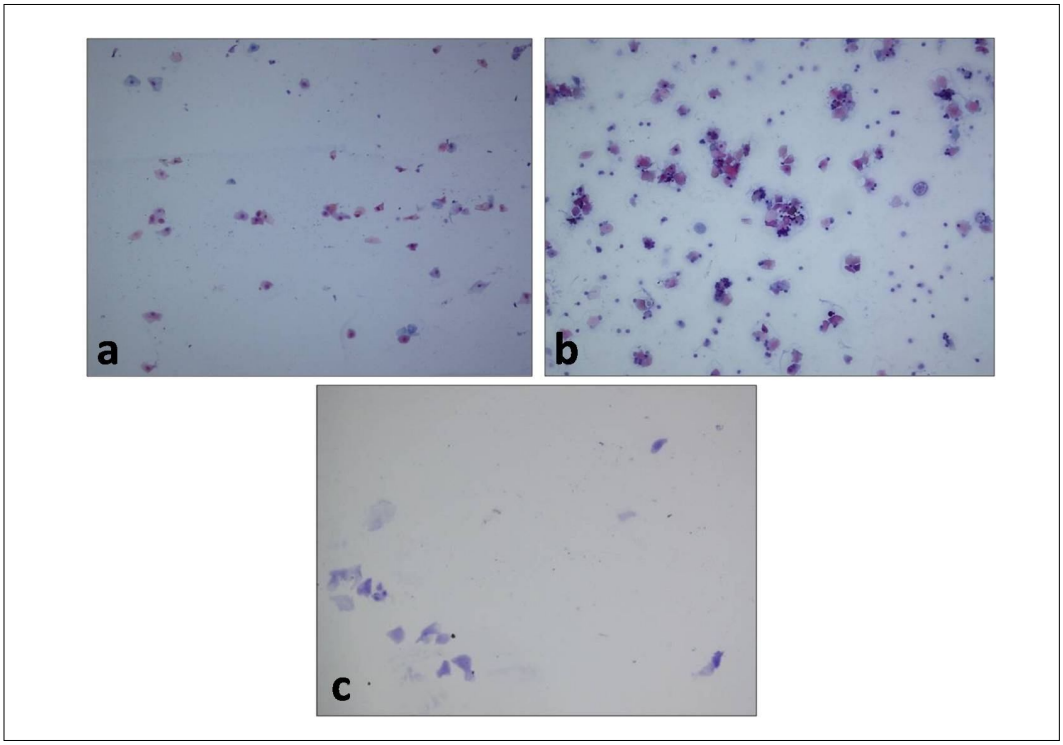

Fig. 1. Nucleated epithelial cells forming clusters and mucinous secretory cells in proestrous phase (Toluidine blue, x100) (a). Cornified cells forming clusters and neutrophilic infiltration between these clusters (Toluidine blue, x100) (b). Thin vaginal epithelium is typical for diestrous phase (Toluidine blue, x100) (c)

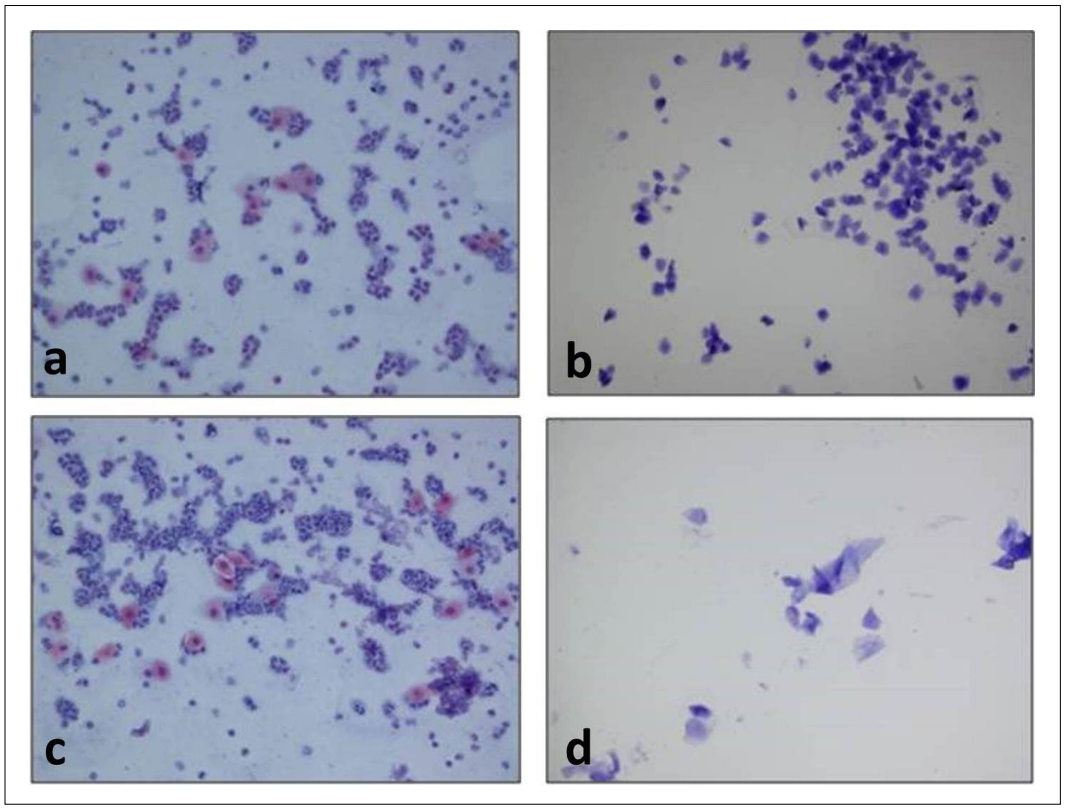

Fig. 2 a-d. Views of proestrous (a); estrous (b); metestrous (c) and diestrous (d) phases after a 30-day exposure to cadmium. Cluster formations of nucleated epitelial cells at proestrous phase is noteworthy

Ninety-day exposure to cadmium chloride resulted in histopathological changes similar to those in study group 2 (Figs. 4a-d). In Figure 4, histopathological views at proestrous (4a), estrous (4b), metestrous (4c) and diestrous (4d) phases after a 90-day exposure to cadmium chloride are shown.

Biochemical Results

As seen in Table 1, estradiol levels were significantly lower in study group 3 that had been exposed to cadmium for 90 days. No statistically significant difference was detected between control groups on estradiol levels (p=0.270). Comparison of study groups regarding estradiol levels revealed that there was remarkable difference between study group 3 and study groups 1 and 2 (p=0.001). No statistically significant difference was detected between groups on FSH, LH and prolactin. The values are respectively 0.05 mIU/mL to FSH, 0.01 mIU/mL to LH and 0.6 ng/mL to prolactin for all control and study groups.

Table 1: Mean estradiol levels in study and control groups

	Study 1 mean±SD (min-max)	*Study 2 mean±SD (min-max)*	*Study 3 mean±SD (min-max)*	*Study 4 mean±SD (min-max)*	*Study 5 mean±SD (min-max)*	*Control 3 mean±SD (min-max)*
Estradiol (pg/mL)	27.67±5.55 (20-35)	31.78±3.31 (25-35)	11.86±2.48 (10-16)	29.17±8.80 (22-34)	24.83±3.37 (19-29)	27.33±1.51 (26-29)

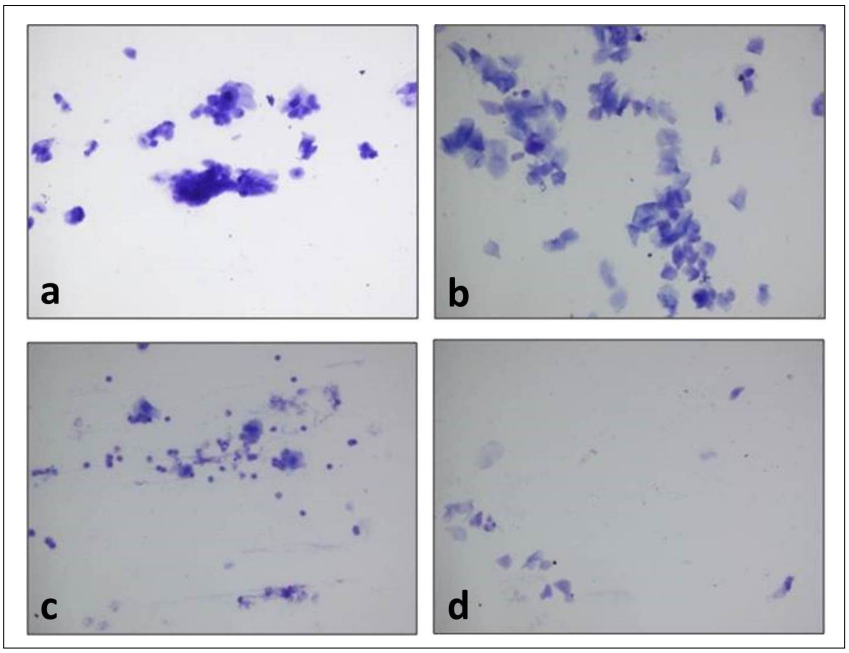

Fig. 3 a-d. Views of proestrous (a); estrous (b); metestrous (c) and diestrous (d) phases after a 60-day exposure to cadmium. Increase in the number of apoptotic cells in all phases is obvious compared to that of study group 1

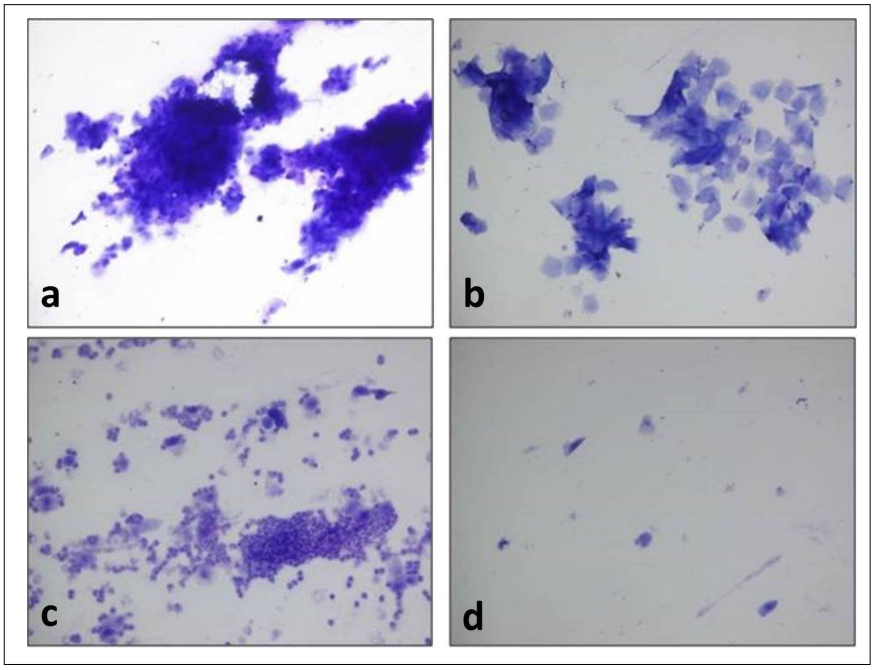

Fig. 4 a-d. Histopathological views at proestrous, estrous, metestrous and diestrous phases after a 90-day exposure to cadmium chloride

Mice in study groups displayed aggressive behavior as time passed by after the initial administration of cadmium chloride. Such a tendency was not observed in the control group.

DISCUSSION

This study was performed to observe the effects of cadmium on fertility parameters in mice. The results have shown that cadmium may contribute to unexplained female infertility and precautions must be taken to minimize occupational cadmium exposure in nursing practice.

Impacts of cadmium on kidney, larynx, liver, thyroid gland and male infertility have been studied in the literature, but there is scarce data on the effects on female infertility (Belani 2014; Rzymski 2015). Distinct from similar studies, the researchers have focused on the possible relevant results of long-term cadmium exposure. From this point of view, the researchers hope that the results will make a unique and original contribution to medical literature.

Interpretation of the histopathological results indicates that even though 30-day exposure does not yield any notable changes in cellular profiles, 60-day and 90-day exposures seem to cause an obvious increase in apoptotic cells in all phases. Nampoothiri et al. (2006) demonstrated that exposure to cadmium resulted in a decrease in activity of gonadotropins together with increased levels of free oxygen radicals and decreased in glutathione. These processes were assumed to be the major contributory mechanisms for infertility.

Paksy et al. (1996) postulated that infertility due to cadmium exposure in mice was linked with irreversible hypophysial dysfunction. Cadmium may inhibit two important regulatory enzymes, steroidogenic acute regulatory protein and cytochrome p450 side chain cleavage and interfere with progesterone synthesis in ovarian granular cells (Zhang and Jia 2007; Dechanet 2011). Moreover, exposure to cadmium resulted in disruption of normal cellular morphology due to lysosomal vesiculation, nuclear chromatin accumulation and mitochondrial changes (Helmestam 2010; Dechanet 2011).

Cadmium was associated with apoptosis at concentrations of 10-30 ìM, and this effect was attributed to increasing in lipid peroxidation and reactive oxygen species in parallel to a diminution of antioxidant systems such as catalase, glutathione and glutathione peroxidase (Duru 2000). The relationship between both male and female infertility and oxidative stress has been clearly identified in many publications (Walsh 2000; Sikka 2001). Cadmium has exhibited effects like estrogen via estrogen receptors and therefore, cadmium has been termed as a potent invivo non-steroidal estrogen agonist (Johnson 2003). Cadmium inhibits steroidogenesis in ovarian tissue by blocking the binding of FSH and LH on granulosa cells (Priya 2004).

Massanyi et al. (2007) have documented that cadmium caused deleterious alterations in cellular morphology and led to edematous changes in vascular walls in a uterus, ovaries, and ovarian tubes. Follicular sizes were found to be decreased, and a number of atretic follicles were significantly higher in mice exposed to cadmium. Interestingly, prolactin levels were increased after exposure of endometrial stromal cells to cadmium (Tsutsumi 2009).

In the present study, analysis of serum hormone levels indicated that there were no differences in levels of FSH, LH and prolactin levels in study and control groups. This may ensource from the usage of human ELISA kits and this constitutes a limitation for the present study. However, long-term (90-day) exposure to cadmium was found to decrease the levels of estradiol significantly. This result is remarkable since decreased estradiol levels may play a role in the pathophysiology of female infertility.

Similar to the results, publications in the literature suggest that exposition of cadmium did not cause any change in levels of FSH, LH and prolactin. Some reports on alterations of serum estradiol levels due to exposure to cadmium at various doses are consistent with the results of the current study (Mortada 2004; Wood 2007). Piasek et al. (1994) stated that exposure to cadmium during pregnancy was not associated with any changes in progesterone levels.

Patients with unexplained infertility must be carefully evaluated regarding environmental exposure to heavy metals and increased social awareness on this topic must be provided. Hazardous impacts of cadmium and other heavy metals on reproductive health must be remembered to promote public health standards. Another important message to be taken from the present study is that integration of experimental research into the nursing discipline is crucial for meeting the needs of current scientific development.

CONCLUSION

In conclusion, the researchers' results have shown that cadmium may contribute to unexplained female infertility. Cessation of smoking must be encouraged, and precautions must be taken to minimize occupational cadmium exposure in nursing practice.

RECOMMENDATIONS

The aim of the present study was to evaluate the effects of cadmium administration on female infertility parameters in mice. Prolonged exposure to cadmium was associated with significantly decreased serum levels of estradiol. Cessation of smoking must be encouraged, and precautions must be taken to minimize occupational cadmium exposure in nursing practice.

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