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Ovarian Toxicity Induced by Dietary Cadmium in Hen

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Abstract To investigate the toxicity of cadmium (Cd) on female reproduction in birds, this study was conducted to determine the changes in biochemical parameters of serum and ovary tissue caused by dietary cadmium in hens. Ninety 50-day-old hyline white hens were randomly divided into three groups (30 hens per group): a control group was fed with basal diet, a low dose group was fed with basal diet containing 140 mg/kg $CdCl_2$ and a high dose group was fed with basal diet containing 210 mg/kg CdCl2. After being treated with Cd for 20, 40 and 60 days, ovary and serum samples were collected and examined for Cd content, histological evaluations, malondialdehyde (MDA) content, glutathione peroxidase (GPx) content, activities of superoxide dismutase (SOD), nitric oxide (NO) content, nitric oxide synthase (NOS) activity, and serum estradiol and progestogen levels. The results showed that the content of Cd, MDA, NO and the activity of NOS in ovary and serum were increased ($P<0.05$), while the level of GPx and the activity of SOD were decreased ($P<0.05$) in low dose and high dose groups. A time- and dose-dependent correlation was observed between serum and ovary tissue cadmium levels. The number of apoptotic cells in the ovary was increased in the Cd treatment group ($P<0.05$). Extensive damage was observed in the ovary. The level of estradiol and progestogen in the serum of low dose and high dose groups was decreased significantly ($P<0.05$). It indicated that Cd exposure resulted in oxidative damage of hens' ovary tissue by altering antioxidant defense enzyme systems, lipid peroxidation, apoptosis and endocrine disturbance which may be possible underlying reproductive toxicity mechanisms induced by Cd.

Keywords Cadmium · Oxidative stress · Apoptosis · Endocrine disturbance · Ovary

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Introduction

Heavy metal cadmium (Cd) is one of the most common environmental pollutants associated with many modern industrial processes. Exposure to Cd is usually the result of environmental contamination by waste from human activities such as the residues found in waste, those released by the combustion of fossil fuels and industry, and the runoff from agricultural land [1, 2]. Relatively large quantities of Cd are found in commercial phosphate fertilizer, thus the increase of Cd contents in soil and plant may result in increase in dietary Cd. So, it is a common finding that poultry industry is affected by Cd toxicity. Body burdens of Cd already approach values considered as critical for the functional impairment of the target organs.

It has been shown that the maximum tolerable dietary Cd level for domestic animals is 0.5 ppm. Dietary concentrations of 1 ppm result in undesirable effects, while 5 ppm cause adverse health effects [3]. Uyanik et al. also found that 50, 75 and 100 mg/kg $CdCl_2$ had adverse effects on biochemical parameters and resulted in tissue accumulation in broilers. It was revealed that cadmium has the potential to induce hepatotoxicity and nephrotoxicity, and supplementation with *E. officinalis*, vitamin E and polyherbal formulation

(stressroak) has a beneficial role in preventing the adverse effects in broilers [4]. Cd is a powerful inducer of oxidative stress. It causes ventral body wall defects in chick embryos treated at Hamburger–Hamilton stages 16–17 [5] and testicular toxicity induced by dietary cadmium in cocks [6].

Many researchers have investigated the reproductive toxicity induced by Cd in mammals. Recent research indicates that Cd is an endocrine disrupter (ED) with detrimental effects on mammalian reproduction [7, 8]. Cd exposure can cause changes in gonadal tissue histopathology, increase oxidative stress, endocrine disruption and apoptosis in rodents, rabbit, sheep and humans [9–11]. Moreover, high doses of Cd could cause rapid and long lasting damage in the testes of male rats [12]. Amara [13] found that Cd treatment led to a decrease in testicular and plasma testosterone levels. Increasing evidence demonstrates that Cd has an apoptotic effect in germ cells of mammals or rodents. Acute Cd exposure resulted in germ cell $CdCl_2$ (2.0 mg/kg) apoptosis in testes [14].

Cd administration could affect the reproductive system in female mammals. Cd administration to rats alters ovarian steroidogenesis, associated with a reduction in progesterone secretion in a dose- and age-dependent manner [9]. Similarly, exposure of cultured rat and human ovarian granulosa cells to Cd caused a reduction in progesterone production [15, 16]. Cd, at concentrations as low as 1 μM, significantly decreased the germ cell density in human fetal ovaries, and Cd increased female germ cell apoptosis [17].

Although many researchers have investigated the toxicity of Cd on mammals and birds, few attempts have been performed on the toxicity of reproduction in hens. Therefore, this research aimed to investigate ovarian toxicity induced by Cd in hens after a subchronic exposure to Cd by a dietary route. The changes in histopathology, oxidative stress, endocrine disruptors and apoptosis were evaluated.

Materials and Methods

Animal Model and Tissue Collection

All procedures used in this experiment were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University. The dietary regimes and the design of dosing have been described previously [18]. Ninety 50-day-old hyline white hens were randomly divided into three groups (30 hens per group): a control group was fed with basal diet, a low dose group was fed with basal diet containing 140 mg/kg $CdCl_2$ and a high dose group was fed with basal diet containing 210 mg/kg CdCl2. The hens were given free access to standard feed and water which were in line with the national standards for feed hygiene (GB 13078-2001 (Cd content in feeds ≤ 0.201 mg/kg, Cd content in water ≤ 0.002 μg/ml)). Ten hens were selected from each group and were killed by decapitation, respectively, at the 20th, 40th and 60th days after Cd exposure. Then, the ovary tissue and serum were collected, respectively, for the detection.

The Content of Cd Assay in Serum and Ovary

The content of Cd in serum and ovary was detected by flame atomic absorption spectrophotometry (FAAS, Shanghai Huipu Analytical Instruments Company). The optimal operating conditions were: wave length 228.8 λ/nm, slit 0.2 nm, burner height 5.0 mm, light current 2.0 I/mA, acetylene discharge 1.5 l/min and air discharge 6.0 l/min. The wet tissue samples were cut into small pieces (1.0 g) with a stainless steel knife and were transferred into beakers. In the digestion procedures, the beakers were added concentrated HNO_3/HCl (4:1) and warmed in a low temperature electric hot plate to solution transparence. The samples were metered volume to 10 ml by 0.5% HNO_3 and measured by FAAS. The content of Cd was calculated by a drawn standard curve.

Histological and Ultrastructural Observations

Specimens were fixed in 10% buffered neutral formalin, processed for paraffin wax sectioning of about 5 μm thickness and stained with hematoxylin and eosin for light microscopy. For the ultrastructural examination, the ovary tissues (size: 1.0 mm × 1.0 mm × 1.0 mm) were fixed immediately in 2.5% glutaraldehyde phosphate buffer saline (v/v, pH 7.2), postfixed in 1% osmium tetroxide (v/v) and stained with 4.8% uranyl acetate following dehydration. The samples were washed in propylene oxide and impregnated with epoxy resins. The semifine sections were contrasted with uranyl acetate and lead citrate for study via microscopy. The microphotographs were taken with a transmission electron microscope (TEM).

Determination of Apoptosis in Ovary

Apoptotic nuclei in tissue sections were identified using the in situ terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) biotin nick-end labeling (TUNEL) technique that identifies DNA strand breaks by labeling their free 3′-OH termini. We used an in situ cell death detection kit (Roche Diagnostics GmbH, Mannheim, Germany). The method distinguishes apoptotic cells from those undergoing necrosis because damaged DNA in the former leads to a different distribution of staining and nuclear morphology. Paraffin wax-embedded tissue sections were treated with proteinase K and the endogenous peroxidase activity was blocked with hydrogen peroxide. The

sections were incubated at 37°C with the terminal TdT nucleotide mixture for 1 h. Then, the reaction was stopped and the slides were rinsed with phosphate-buffered saline. Nuclear labeling was developed with horseradish peroxidase and diaminobenzidine. Hematoxylin was used for counterstaining. Quantitative evaluation of the apoptotic index was performed by manual counting of positively stained nuclei at 400× magnification.

Apoptosis was determined in five ovaries from each group of hen by counting at least 1,000 cells from five to six sections of each ovary. The results are expressed as the percentage of TUNEL-positive cells among the total number of cells counted.

Measurement of Oxidative Stress

The content of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) was assayed by using kits (Nanjing Jiancheng Bioengineering Institute, PR China) according to the manufacturer's protocol.

Determination of NO and NOS in Serum and Ovary

The nitric oxide (NO) and nitric oxide synthase (NOS) levels of ovary and serum were measured using the commercial kits (Jiancheng Institute of Biotechnology, Nanjing, China). NO scontent were expressed as micromole per milligram of protein. Data were expressed as units of NOS activity per milligram of protein.

Determination of Estradiol and Progestogen Level in Serum

Serum estradiol and progestogen were determined by radioimmunoassay (RIA) using estradiol and progestogen 125I RIA kit, respectively, (Beijing North Institute of Biological Technology, PR China) according to the manufacturer's protocol. Radioactivity was determined using an automatic gamma counter. All samples were run in duplicate in a single assay to avoid interassay variation.

Statistical Analysis

Statistical analysis of all data was performed with SPSS 13.0 computer software and all data were assessed by one-way ANOVA. All data were expressed as means ± SD. $P<0.05$ was considered significant difference.

Results

The Content of Cd

Cd content in the ovary tissue and serum were shown in Table 1. A time- and dose-dependent correlation was observed between serum and ovary tissue cadmium levels after treatment with Cd for 20, 40 and 60 days. Compared with the corresponding control group, Cd content in low dose and high dose groups increased significantly ($P<0.05$) in ovary tissue and serum at each time point. Compared with the corresponding low dose group, Cd content in the high dose group increased significantly ($P<0.05$) in ovary tissue and serum at each time points.

Histopathology and Ultrastructure

Histology of the ovary in control hens showed normal ovarian structure with regular morphology and normal oogenesis (Fig. 1 a1, a2). Hens treated with Cd showed ovarian lesions with severe necrosis and degeneration of ovarian follicle and interstitial cell (Fig. 1 a3, a4, a5, a6). In addition, ovarian damage increased with the treatment of Cd, especially the high concentration of Cd.

Electron microscopy showed normal ovarian ultrastructure in the control group (Fig. 2 a1, b1). Cd treatment caused extensive ovarian tissue damage. Growing follicle displayed morphological characteristics of apoptosis including markedly swollen mitochondria with degeneration or loss of cristae, dilated cisternae of the smooth endoplasmic reticulum (SER), blebbing of the membranes with cytoplasmic vacuolation, cell shrinkage and chromatin condensation (Fig. 2 a2,a4). Chromatin condensation showed as crescentic bodies with a clear boundary of the nuclear membrane (Fig. 2 a3). Mitochondria were markedly swollen with cristae degeneration, or fuzzy and dilated cisternae of the SER were observed in interstitial cells (Fig. 2 b3, b4). Interstitial cells showed chromatin condensation and margination (Fig. 2 b2, b4).

Table 1 Cd contents in serum and ovary

Parameters	Sample	Group	20 days	40 days	60 days
Cd	Ovary (μg/g)	Control	0.003 ± 0.003^{Cc}	0.165 ± 0.049^{Bc}	0.326 ± 0.081^{Ac}
		Low dose	1.350 ± 0.051^{Cb}	1.543 ± 0.069^{Bb}	2.401 ± 0.051^{Ab}
		High dose	2.427 ± 0.049^{Ca}	2.796 ± 0.060^{Ba}	3.334 ± 0.049^{Aa}
	Serum (μg/ml)	Control	1.280 ± 0.411^{c}	1.602 ± 0.612^{c}	1.641 ± 0.446^{c}
		Low dose	5.177 ± 0.389^{Bb}	7.794 ± 0.567^{Ab}	8.833 ± 0.421^{Ab}
		High dose	10.369 ± 0.772^{Ba}	11.322 ± 0.316^{ABa}	13.199 ± 0.446^{Aa}

Values are expressed as mean ± SD, $n=5$. Means with different uppercase letters within rows are significantly different ($P<0.05$) and means with different lowercase letters within columns are significantly different ($P<0.05$)

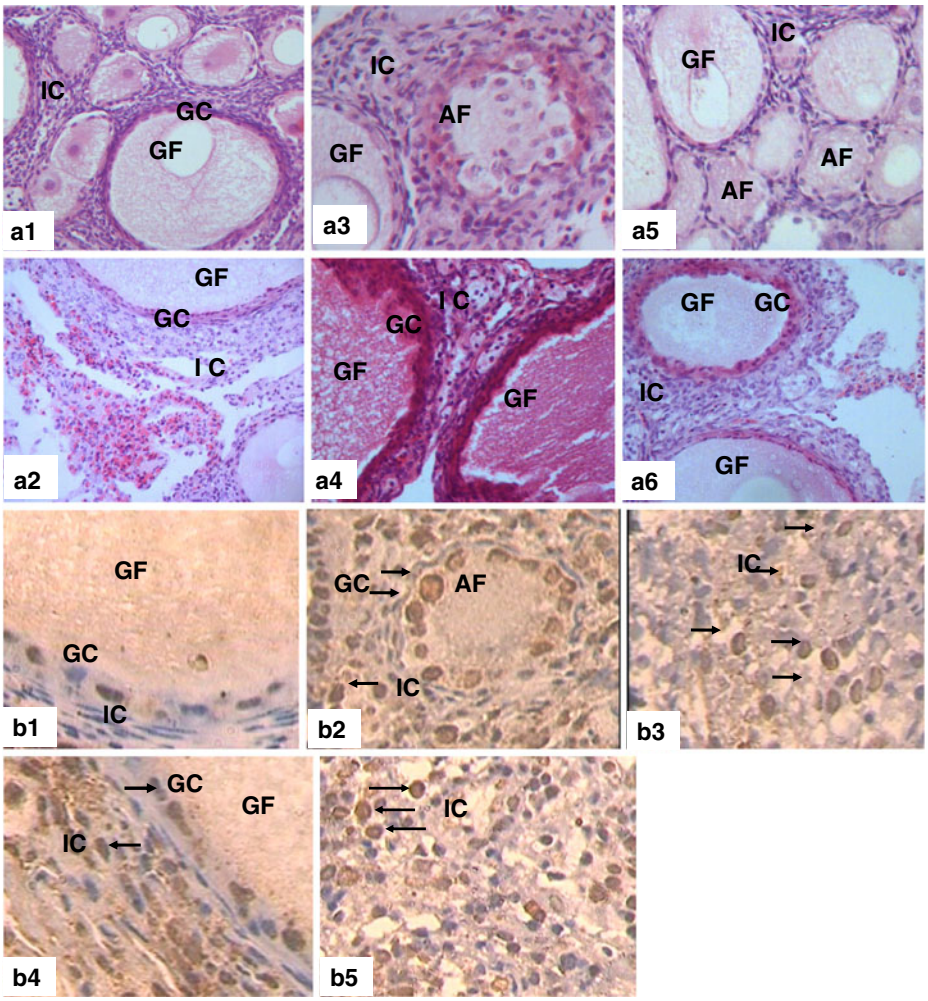

Fig. 1 Histology (**a** hematoxylin and eosin staining) and TUNEL staining (**b** counterstained with hematoxylin) of the ovarial tissues of hen in different groups at the end of the 60 days of treatment with Cd. *a1* Section of ovaries from a hen in the control group. *a2* Section of ovaries from a hen in the control group. *a3* Growing follicle of a hen ovaries from the low dose group. *a4* Section of ovaries from a hen in the low dose group. *a5* Growing follicle of a hen ovaries from the high dose group showing a high level of atretic follicle. *a6* Section of ovaries from a hen in the high dose group. Many interstitial cells were analosis, but are undergoing degeneration along with loss of oogenesis. *b1* Normal ovaries in the control groups. *b2* Granular cell of a hen's ovaries from the low dose group showing a high level of apoptosis (*arrows*). *b3* Interstitial cell of a hen's ovaries from the low dose group showing increased numbers of apoptotic cells (*arrows*). *b4* Granular cell of a hen's ovaries from the high dose group showing a high level of apoptosis (*arrows*). *b5* Interstitial cell of a hen's ovaries from the high dose group showing increased numbers of apoptotic cells (*arrows*). *GF* growing follicle, *AF* atretic follicle, GC granular cell, *IC* interstitial cell. *a1*×100, *a2*×400, *a3*×100, *a4*×400, *a5*×100, *a6*×400, *b1*×400, *b2*×400, *b3*×400, *b4*×400, *b5*×400

Apoptosis in the Ovary

The number of apoptotic cells in the ovary was significantly increased in low dose and high dose groups compared with the corresponding control group. Interestingly, most of the apoptotic cells in the ovary were found in the granulosa cells and interstitial cells of ovarian region (Fig. 1 b2, b3, b4, b5). Effects of Cd treatment on the apoptosis index in the ovary were presented in Fig. 3. The number of apoptotic cells in the Cd group significantly increased compared with the corresponding control group ($P<0.05$). In addition, the apoptosis index increased with the treatment of Cd, which was higher in the low dose group at the 40th and the 60th days than in the high dose group.

Variation in Antioxidant Activity

As shown in Table 2, the MDA level in the ovary of low dose and high dose groups was significantly higher than in the corresponding control group ($P<0.05$). The MDA level of the ovary increased with the treatment of Cd. The MDA level in the serum of low dose and high dose groups was significantly higher than the corresponding control group ($P<0.05$), and the MDA level in the high

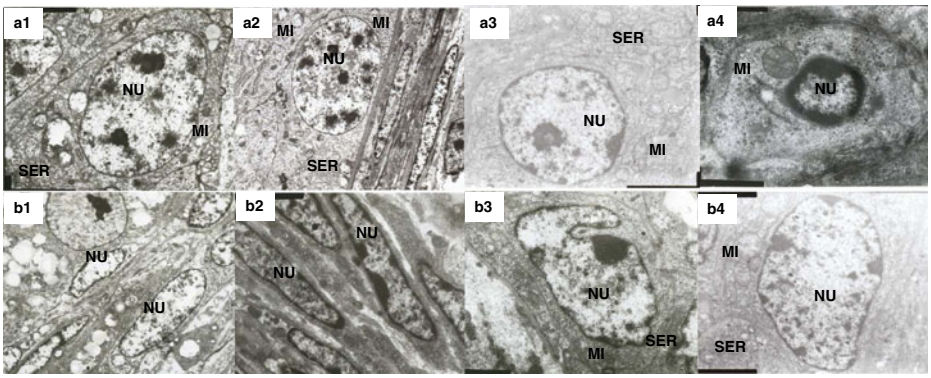

Fig. 2 **a** Transmission electron microscopy of ovarial tissues. *a1* The control showed normal granular cell. *a2* Granular cells in the low dose group had markedly swollen mitochondria with degenerated or missing cristae, dilated smooth endoplasmic reticulum (SER) cisternae and nuclear envelope resolved. *a3* Granular cells in the low dose group displayed morphological characteristics of apoptosis including cell shrinkage and chromatin condensation showing as crescentic bodies with a clear boundary under the nuclear envelope. *a4* Granular cells in the high dose group had markedly swollen mitochondria with degenerated or missing cristae, dilated SER cisternae and nuclear chromatin concentrated under the caryotheca. **b** Transmission electron microscopy of ovarial tissues. *b1* The control showed normal interstitial cells. *b2* Interstitial cells in the low dose group had markedly swollen mitochondria with loss of cristae and nuclear chromatin condensation. *b3* Interstitial cells in the low dose group showed slightly dilated SER cisternae, mitochondria with degenerated or missing cristae and nuclear chromatin condensation. *b4* Interstitial cells in the high dose group had markedly swollen mitochondria with loss of cristae and nuclear chromatin concentrated under the caryotheca. *SER* smooth endoplasmic reticulum, *MI* mitochondria, *NU* nucleus. *a1*×8,000, *a2*×10,000, *a3*×15,000, *a4*×10,000, *b1*×2,000, *b2*×8,000, *b3*×12,000, *b4*×10,000

dose group increased more obviously than the low dose group ($P<0.05$).

The SOD and GPx activities in the ovary and serum were shown in Table 2. The activities of SOD and GPX decreased compared with the corresponding control group. A more significant decrease of SOD and GPx activities could be observed the longer the treatment and the higher the concentration of Cd. Especially at the end of the 40th and the 60th days, the differences were significant in both ovary and serum ($P<0.05$). The SOD and GPx activities in the high dose group decreased more obviously than the low dose group ($P<0.05$).

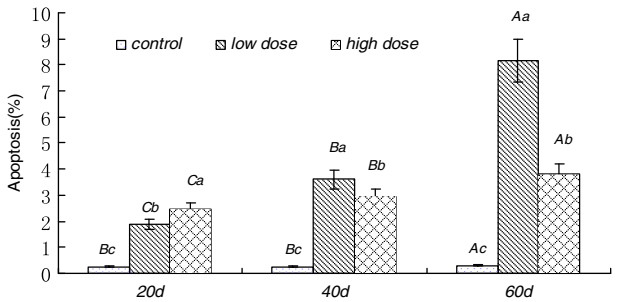

Fig. 3 Effects on ovary cell apoptosis in Cd-treated hens. Each value is the mean ± SD of at least five hens. Statistically significant differences: means with different *uppercase* within the same group at different time points are significantly different ($p<0.05$) and means with different *lowercase* in different groups at the same time point are significantly different ($p<0.05$)

Variation of NO Metabolism

The NO levels and NOS activity in the ovary and serum were shown in Table 3. NO levels and NOS activity significantly increased in the ovary and serum (at 20 days, 40 days and 60 days) of Cd treatment groups compared with the corresponding control groups ($P<0.05$). NO levels and NOS activity increased with the treatment of Cd, especially in the high dose group.

Effects of Cd on Estradiol and Progestogen Levels in Serum

Effects of Cd on estradiol and progestogen levels in serum were showed in Table 4. The concentrations of both estradiol and progestogen increased in the control group. At the end of the 20th, 40th and 60th days, significant decreases were observed in the concentrations of both estradiol and progestogen in the Cd group compared with the corresponding control group ($P<0.05$). The more Cd added in the feed, the lower was the concentration of both estradiol and progestogen in the serum, and there is a time- and dose-dependent correlation.

Discussion

In this study, we showed that the content of Cd in the ovary and serum was increased after treatment with Cd. Tissue Cd content was consistent with serum Cd, but the increase of

Table 2 Effects of antioxidant abilities in serum and ovary

Parameters	Sample	Group	20 days	40 days	60 days
MDA	Ovary (nmol/mg protein)	Control	2.29 ± 0.42^{b}	2.46 ± 0.16^{b}	2.72 ± 0.12^{b}
		Low dose	2.98 ± 0.38^{Ba}	3.49 ± 0.22^{ABa}	3.85 ± 0.47^{Aa}
		High dose	3.27 ± 0.10^{Ca}	3.79 ± 0.20^{Ba}	4.45 ± 0.36^{Aa}
	Serum (nmol/ml)	Control	4.52 ± 0.61^{c}	4.89 ± 0.31^{c}	4.95 ± 0.28^{c}
		Low dose	6.69 ± 0.41^{Cb}	7.63 ± 0.33^{Bb}	8.76 ± 0.28^{Ab}
		High dose	8.07 ± 0.60^{Ca}	9.70 ± 0.31^{Ba}	12.17 ± 0.56^{Aa}
SOD	Ovary (NU/mg protein)	Control	35.79 ± 1.17^{a}	32.90 ± 1.32^{a}	32.60 ± 1.69^{a}
		Low dose	34.01 ± 0.79^{Aa}	30.28 ± 0.87^{Bb}	29.64 ± 0.71^{Bb}
		High dose	32.02 ± 1.19^{Ab}	28.64 ± 0.72^{Bc}	27.10 ± 0.38^{Bc}
	Serum (NU/ml)	Control	203.99 ± 0.76^{a}	199.82 ± 1.48^{a}	198.11 ± 1.11^{a}
		Low dose	199.86 ± 1.51^{Ab}	196.31 ± 0.92^{Ab}	184.87 ± 1.53^{Bb}
		High dose	197.74 ± 1.87^{Ab}	191.05 ± 1.27^{Bc}	177.22 ± 1.77^{Cb}
GPX	Ovary (NU/mg protein)	Control	10.19 ± 0.77^{a}	10.41 ± 0.47^{a}	11.99 ± 0.85^{a}
		Low dose	8.14 ± 0.59^{Ab}	7.08 ± 1.20^{Ab}	5.43 ± 0.78^{Bb}
		High dose	7.52 ± 1.14^{Ab}	5.84 ± 1.60^{Bb}	4.40 ± 0.84^{Bb}
	Serum (NU/ml)	Control	191.85 ± 10.74^{a}	195.37 ± 16.18^{a}	197.61 ± 5.49^{a}
		Low dose	173.68 ± 10.34^{Aa}	143.60 ± 12.79^{Bb}	124.83 ± 10.41^{Bb}
		High dose	169.85 ± 4.61^{Ab}	127.14 ± 5.58^{Bc}	94.06 ± 8.42^{Cb}

Values are expressed as mean ± SD, $n=5$. Means with different uppercase letters within rows are significantly different ($P<0.05$) and means with different lowercase letters within columns are significantly different ($P<0.05$)

ovary tissue Cd content was lower compared with serum Cd. Therefore, it showed that Cd did not preferentially accumulate in ovary tissue in hens. The toxicity of Cd on the genitical gland varied in different animals mainly because of the distinction of its accumulation in different tissues. Cd has a long biological half-life and accumulates over time in kidney, liver and cerebellum as well as in reproductive organs including placenta, testis and ovaries [19, 20]. Administration of Cd could induce histological changes in the increased occurrence of primary atretic follicles that indicated alterations in the membrane structures and organelles of oocytes and in the follicular cells of the stratum granulosum in sheep ovarian [11]. Cd has also been shown to increase oocyte degeneration rate and impair

Table 3 Effects on NO levels and NOS activities in serum and ovary

Parameters	Sample	Group	20 days	40 days	60 days
NO	Ovary (μmol/mg protein)	Control	0.53 ± 0.01^{b}	0.53 ± 0.02^{c}	0.62 ± 0.02^{c}
		Low dose	0.71 ± 0.06^{a}	0.76 ± 0.02^{b}	0.79 ± 0.02^{b}
		High dose	0.83 ± 0.08^{Ca}	0.93 ± 0.09^{Ba}	1.16 ± 0.19^{Aa}
	Serum (μmol/l)	Control	11.19 ± 0.91^{b}	11.32 ± 0.84^{c}	11.91 ± 0.79^{c}
		Low dose	11.63 ± 0.49^{Bb}	13.39 ± 1.39^{ABb}	15.03 ± 1.23^{Ab}
		High dose	15.71 ± 0.50^{Ba}	16.85 ± 1.08^{Ba}	22.11 ± 0.82^{Aa}
NOS	Ovary (NU/mg protein)	Control	4.44 ± 0.67^{b}	5.22 ± 0.46^{b}	5.47 ± 0.96^{b}
		Low dose	5.34 ± 0.61^{Bb}	6.72 ± 0.55^{Aa}	7.98 ± 0.98^{Aa}
		High dose	7.08 ± 0.62^{Ba}	8.05 ± 1.07^{ABa}	9.67 ± 1.28^{Aa}
	Serum (NU/ml)	Control	30.64 ± 3.34^{b}	32.59 ± 2.09^{b}	33.76 ± 1.33^{c}
		Low dose	34.77 ± 2.86^{a}	35.65 ± 1.72^{a}	37.20 ± 1.88^{b}
		High dose	36.19 ± 2.23^{Ba}	38.97 ± 3.08^{ABa}	40.84 ± 1.87^{Aa}

Values are expressed as mean ± SD, $n=5$. Means with different uppercase letters within rows are significantly different ($P<0.05$) and means with different lowercase letters within columns are significantly different ($P<0.05$)

Table 4 The content of estradiol and progestogen in serum

Sample	Parameters	Group	20 days	40 days	60 days
Serum	Estradiol (pmol/l)	Control	79.82 ± 4.17^{Ca}	86.48 ± 3.09^{Ba}	93.17 ± 3.84^{Aa}
		Low dose	68.08 ± 5.07^{Ab}	62.66 ± 2.54^{Ab}	58.55 ± 2.98^{Bb}
		High dose	61.18 ± 3.28^{Ab}	58.82 ± 3.02^{Ab}	47.77 ± 2.29^{Bc}
	Progestogen (nmol/l)	Control	212.36 ± 4.09^{Ba}	224.82 ± 9.05^{Ba}	238.78 ± 3.17^{Aa}
		Low dose	191.96 ± 4.97^{Ab}	185.16 ± 3.23^{Bb}	165.62 ± 2.01^{Cb}
		High dose	152.92 ± 3.56^{Ac}	148.2 ± 3.29^{Bc}	139.9 ± 3.70^{Cc}

Values are expressed as mean ± SD, $n=5$. Means with different uppercase letters within rows are significantly different ($P<0.05$) and means with different lowercase letters within columns are significantly different ($P<0.05$)

oocyte maturation in sheep [21] and pigs [10]. Similarly, adult male rats have been shown to develop gonadal damage following administration of Cd [12]. Our results demonstrated that Cd exposure led to morphological changes in ovary of hens (atrophy, necrosis and degeneration of ovarian follicle and interstitial cell) (Fig. 1 a3–6), which was in accordance with the results in mammals and rodents [11, 19].

Various damages of Cd on germ cell have been described, but definitive conclusions about its actions depend on the experimental model and the dosage [22]. Cd exposure increase the numbers of apoptotic cells in the testes of cocks, as determined by terminal dUTP nick-end labeling (TUNEL) staining [6]. A most recent single dose (sc) of Cd chloride treatment study in rat found that Cd-induced apoptosis of germ cell at low doses of Cd (0.13 and 0.15 mg/100 g body weight [BW]), while high doses of Cd (0.2 and 0.3 mg/100 g BW) caused more necrosis than apoptosis [22]. Our results showed that the number of apoptotic cells in low dose and high dose groups significantly increased compared with the control group. The apoptosis index increased with the treatment of Cd, which was higher in the low dose group at the 40th and 60th days than in the high dose group. The apoptosis was found mainly occurring in ovarian tissue in the low dose group (140 mg/kg) which was consistent with previous studies [22].

Various effects of Cd on reproductive endocrinology have been described, but definitive conclusions about its actions on target tissues vary depending on the experimental model and the dosage employed [23, 24]. In vitro studies showed that Cd treatment impaired testosterone production in isolated Leydig cells and reduced the biosynthesis of progesterone in cultured human trophoblast cells and ovaries in ovarian granular cells [7, 15, 25, 26]. The disruption of steroidogenesis is likely to be an initial target of Cd toxicity as an endocrine modulator [8, 9, 25]. The present study revealed that Cd exposure decreased serum estradiol and progesterone levels in hens which could be explained by the obvious pathological changes and apoptosis in the ovarian cell components (e.g., growing follicles, ovaries granule cells and interstitial cells). These results suggested that the toxicity of Cd on the reproductive system in birds was in consistence with mammals and rodents.

The molecular mechanism about the toxic effect of Cd is not well understood. Various studies connect Cd with oxidative stress, since this metal can alter the antioxidant defense system and caused marked disturbances of the antioxidant defense system both in vivo and in vitro [27–30]. In the present study, we found that the activities of SOD and GPx in the ovary and serum exposed to Cd decreased, while the MDA content increased significantly compared to the control group in a time- and dose-dependent pattern which suggested that Cd exposure disrupted oxidative stress in hen ovary and serum. The overproduction of ROS was one of the possible mechanisms of the oxidative stress induced by Cd treatment. Our results were in agreement with the previous studies which clearly demonstrated that Cd exposure increases MDA and suppressed the antioxidant defense mechanisms in the ovary and serum, with significant reduction in ovarian function and hormone secretion of genitical gland [11].

In conclusion, our study demonstrated that dietary Cd exposure caused histopathological changes, oxidative stress, endocrine disorder and apoptosis in the ovary of hens. There were significant reductions in ovary function and secretion. Moreover, ovarian damage and atretic follicle increased with the treatment of Cd. However, further investigations are needed to clarify the relationship between Cd toxication and breeding function of birds.

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