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# Effects of maternal cadmium exposure during late pregnant period on testicular steroidogenesis in male offspring

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ABSTRACT

Cadmium (Cd) is a testicular toxicant and endocrine disruptor. In the present study, we investigated the effects of maternal Cd exposure during the late pregnant period on testicular development and steroidogenesis in male offspring. Pregnant mice were injected intraperitoneally with $CdCl_2$ (0.5 mg/kg) daily from gestational day (gd) 13 to gd 17. As expected, fetal weight and crown length were significantly decreased in pups whose mothers were exposed to Cd. Importantly, absolute and relative weights of testes were significantly decreased in male fetuses. In addition, maternal Cd exposure during pregnancy markedly reduced serum T level and downregulated the expression of steroidogenic acute regulatory (StAR) protein, P450scc, P45017α and 17β-HSD in testes of male fetuses. Interestingly, the level of serum and testicular T at adulthood remained decreased in male offspring of Cd-exposed mice. Correspondingly, the expression of testicular P450scc was downregulated in male adult offspring whose mothers were exposed to Cd during pregnancy. Fertility analysis found that the number of live fetuses per litter in F2 generation was significantly decreased in Cd-treated group. Additional experiment showed that placental Cd level was increased about 750 folds in dams injected with Cd. However, only traces of blood Cd was measured in fetuses whose mothers were exposed to Cd during the late pregnant period. Taken together, these results suggest that placenta could deter most of Cd from passing from dams to fetuses. The impairments on testicular steroidogenesis in male offspring could not be attributed to a direct action of Cd on fetal testes.



## 1. Introduction

Cadmium (Cd) is one of major occupational and environmental toxicants. Cd is frequently used in electroplating, pigments, paints, welding, and Ni–Cd batteries. Workers in these occupations are exposed to Cd at significantly higher levels than the general public (Beveridge et al., 2010). The general population is exposed to Cd via drinking water, food and cigarette smoking (Honda et al., 2010). Cd is a well-known carcinogen. It has been suggested that Cd is involved in carcinogenesis in multiple organs including lung, kidney, prostate, liver, pancreas, and testes (Waalkes, 2000; Joseph, 2009). Cd is a reproductive toxicant in human. Increasing evidence demonstrated that environmental exposure to Cd is associated with male infertility and the poor semen quality in humans (Pant et al., 2003). According to several earlier studies, there was a significant inverse correlation between blood Cd level and semen quality (Xu et al., 1993; Telisman et al., 2000). In addition, oxidative DNA damage in human spermatozoa is associated with Cd concentration in seminal plasma (Xu et al., 2003). A recent study indicated that a low level of Cd accumulation in semen might contribute to male infertility by reducing sperm quality (Wu et al., 2008). Cd is a testicular toxicant in experimental animals (Siu et al., 2009). Several studies have demonstrated that Cd induces apoptosis in testicular germ cells (Ozawa et al., 2002; Kim and Soh, 2009). As a well-known endocrine disrupting chemical, Cd is not only a regulator of hypothalamus and pituitary hormone secretion (Lafuente et al., 2003, 2004), but also disrupts testicular testosterone (T) production (Laskey and Phelps, 1991; Ji et al., 2010). Several studies showed that Cd inhibited the expression of testicular steroidogenic acute regulatory (StAR) protein, which is responsible for the rate-limiting step in steroidogenesis (Gunnarsson et al., 2004; Sen Gupta et al., 2004; Ji et al., 2010). In addition, pubertal Cd exposure downregulates the level of cytochrome P450 cholesterol side-chain cleavage (P450scc) enzyme, cytochrome P450 17α-hydroxysteroid dehydrogenase ($P450_{17\alpha}$) and 17β-hydroxysteroid dehydrogenase (17β-HSD), several key enzymes for T biosynthesis in testes (Sen Gupta et al., 2004; Ji et al., 2010).

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On the other hand, Cd, at a high dose, is also a potent teratogen in rodent (Barr, 1973). When administered to mice during gestation, Cd induced malformations of the neural tube, craniofacial region, limbs, trunk, viscera, and axial skeleton in fetuses (Hovland et al., 1999; Scott et al., 2005; Paniagua-Castro et al., 2007; Robinson et al., 2009). A recent in vivo study found that maternal Cd exposure during gestation perturbed the vascular system of the adult rat offspring (Ronco et al., 2011). An in vitro study showed that Cd, at low concentrations, altered the survival of male and female germ cells in humans (Angenard et al., 2010). The purpose of the present study was to investigate whether maternally administered Cd can pass through placental barrier and to explore whether maternal exposure to a low dose of Cd during the late pregnant period on testicular development and steroidogenesis in male offspring.

## 2. Materials and methods

### 2.1. Chemicals

Cadmium chloride ($CdCl_2$) was purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were from Sigma or as indicated in the specified methods.

### 2.2. Animals and treatments

CD-1 mice (8–10 week-old; male mice: 28–30 g; female mice: 24–26 g) were purchased from Beijing Vital River whose foundation colonies were all introduced from Charles River Laboratories, Inc. The animals were allowed free access to food and water at all times and were maintained on a 12-h light/dark cycle in a controlled temperature (20–25 °C) and humidity (50 ± 5%) environment for a period of 1 week before use. For mating purposes, four females were housed overnight with two males starting at 9:00 p.m. Females were checked by 7:00 a.m. The next morning, and the presence of a vaginal plug was designated as gestational day (gd) 0. Thirty-six pregnant mice were divided into two groups. Eighteen pregnant mice were injected intraperitoneally (i.p.) with $CdCl_2$ (0.5 mg/kg) daily during late pregnant period (from gd 13 to gd 17). Our preliminary results showed that preterm labour occurred in some dams when pregnant mice were daily injected with more than 1.0 mg/kg Cd during the late pregnant period. In order to investigate the effects of low dose Cd exposure during the late pregnant period on testicular development and steroidogenesis in more detail, 0.5 mg/kg $CdCl_2$ was administered daily from gd 13 to gd 17 in the present study. Eighteen normal saline (NS)-treated pregnant mice served as controls. Twelve dams each group were sacrificed by decapitation on gd 18, and whole blood was collected in a tube with heparin (250 U) for subsequent determination of total Cd in maternal blood. Placenta were collected for the measurement of Cd. Fetuses were dissected under a binocular microscope. Sex was determined by the morphology of the gonalds. All male fetuses were sacrificed by decapitation. Whole blood was collected in a tube with heparin (250 U) from half male fetuses for the measurement of Cd in fetal blood. Fetal sera were collected from half male fetuses and kept at −20 °C for subsequent determination of T in fetal serum. Fetal testes were aseptically removed from half male fetuses for RT-PCR and immunoblotting. Fetal testes were removed from half male fetuses and immersed in modified Davidson's fluid (mDF) for 6 h for terminal dUTP nick-end labeling (TUNEL) staining and histology. For remaining pregnant mice (six for each group), natural birth occurred between gd 18 at 16:00 and gd 19 at 18:00. Within 24 h after birth, excess pups were removed, so that only eight pups (four males and four females) were kept per dam. At postnatal day (PND) 70, half male pups (two each litter and 12 each group) were slaughtered under thiopental anesthesia, always in the morning. The cauda epididymides were removed for sperm analysis. Testes were excised, dissected, weighted, and then divided in two parts: left one was kept at −80 °C for subsequent measurement of T, RT-PCR and immunoblotting. All the right testes were immersed in mDF for 12–24 h for histology, TUNEL and immunohistochemistry. To investigate the effects of maternal Cd exposure during the late pregnant period on the fertility of adult male offspring, 12 males (two each litter) whose mothers were exposed to Cd during the late pregnant period were housed with untreated females. All procedures on animals followed the guidelines for humane treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

### 2.3. Testicular histology

Two cross-sections from each testis were embedded in paraffin using standard procedures. Paraffin-embedded tissues were serially sectioned. At least two nonserial sections were stained with hematoxylin and eosin (H&E) using standard procedures for morphological analyses. Leydig cells in fetal testes were identified by immunostaining for 3β-hydroxysteroid dehydrogenase (3β-HSD). Sections were mounted onto coated slides, dewaxed, and rehydrated. Antigen retrieval was performed by pressure cooking slides for 5 min in 0.01 M citrate buffer (pH 6.0). Slides were incubated for 30 min in 3% (v/v) hydrogen peroxide in methanol to block endogenous peroxidase activity and then washed in Tris-buffered saline [TBS; 0.05 M Tris, 0.85% (w/v) NaCl (pH 7.4)]. Nonspecific binding sites were blocked with an appropriate normal serum diluted 1:5 in TBS containing 5% (w/v) BSA before the addition of rabbit polyclonal antibody against mouse 3β-HSD (Santa Cruz, USA) and overnight incubation at 4 °C. After washing in TBS, slides were incubated for 30 min with the appropriate secondary antibody conjugated to biotin goat anti-rabbit (Santa Cruz, USA), diluted 1:500 in the blocking mixture. This was followed by 30 min incubation with horseradish peroxidase-labeled avidin–biotin complex (Dako). Immunostaining was developed by application of diaminobenzidine (liquid $DAB^+$; Dako), and slides were counterstained with hematoxylin, dehydrated, and mounted using Pertex mounting medium (Cell Path, Hemel Hempstead, UK). For fetal testes, the number of 3β-HSD-positive cells was counted in three randomly selected fields from each slide at a magnification of 400×. For adult testes, the number of 3β-HSD-positive cells was counted in 12 randomly selected fields from each slide at a magnification of 400×.

### 2.4. Radioimmunoassay (RIA)

Fetal sera were separated by centrifugation and stored at −80 °C until assay for T. serum T was measured using $^{125}$I-based RIA kits from Beijing north institute of biological technology (Beijing, China).

### 2.5. Semiquantitative RT-PCR

Total cellular RNA was extracted using TRI reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer's instructions. RNase-free DNase (Promega, Madison, WI) was used to remove genomic DNA. The integrity and concentration of RNA was determined by measuring absorbance at 260 nm followed by electrophoresis on agarose gels. For the synthesis of cDNA, 4.0 μg of total RNA from each sample was reversely transcribed in a 40 μl reaction volume containing 0.5 μg oligo(dT)15 (Promega, Madison, WI), 1 mM of each dNTP, 15 units AMV RT (Promega), and 1 U/μl recombinant RNasin RNase inhibitor (Promega) in 5 mM MgCl2, 10 mM Tris·HCl, (pH 8.0), 50 mM KCl, and 0.1% Triton X-100. The mixture was heated to 70 °C for 10 min, maintained at 42 °C for 60 min, and then heated to 95 °C for 5 min to terminate the reaction. Reaction mixtures (25 μl) for PCR were assembled using 2 μl cDNA template, 0.625 units Hot Start Polymerase (Promega), 200 μM of each dNTP, 1.5 mM $MgCl_2$, and 1.0 μM of each primer in 1× Flexi buffer. The primers were synthesized by Sangon Biological Technology (Shanghai, China), according to sequence designs previously described. The primer pairs are shown in Table 1. The amplified PCR products were subjected to electrophoresis at 75 V through 1.5% agarose gels (Sigma, St. Louis, MO) for 45 min. The pBR322 DNA digested with Alu I was used for molecular markers (MBI Fermentas). Agarose gels were stained with 0.5 mg/ml ethidium bromide (Sigma, St. Louis, MO) TBE buffer.

### 2.6. Immunoblotting

Samples from fetal testes were homogenized in lysis buffer containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecylsulfate (SDS), and 1 mM phenylmethylsulfonyl fluoride (PMSF). Samples were then centrifuged at 15,000 × *g* for 15 min. Supernatants from each sample were added to a gel loading buffer (100 mM Tris, pH 6.8, 20% glycerol, 200 mM DTT, 4% SDS, 0.03% bromophenol blue) and boiled for 5 min. Proteins (50 μg/sample) in loading buffer were subjected to electrophoresis in 10% SDS–polyacrylamide gel for 3 h. The gel was transferred electrophoretically onto a polyvinylidene fluoride membrane (Immobilon-P; Millipore Corp., Bedford, MA, USA) and blocked in 5% nonfat powdered milk in Dulbecco's PBS (DPBS) overnight at 4 °C. The membranes were then incubated for 2 h with rabbit polyclonal antibody against mouse StAR, $P450_{17\alpha}$, 17β-HSD and P450scc (Santa Cruz Biotechnology, USA) or β-actin (Beijing Biosynthesis Biotechnology, Beijing, China) at room temperature. After washes in DPBS containing 0.05% Tween-20 four times for 10 min each, the membranes were incubated with goat anti-rabbit IgG antibody for 2 h. The membranes were then washed for four times in DPBS containing 0.05% Tween-20 for 10 min each, followed by signal development using an enhanced chemiluminescence (ECL) detection kit from Pierce (Pierce Biotechnology, Rockford, IL).

### 2.7. Terminal dUTP nick-end labeling (TUNEL) staining

For the detection of apoptosis, paraffin-embedded sections were stained with the TUNEL technique using an in situ apoptosis detection kit (Promega) according to the manufacturer's protocols. For fetal testes, the number of TUNEL-positive cells was counted from each slide at a magnification of 400×. For adult testes, the number of TUNEL-positive cells in testes was counted in 12 randomly selected fields from each slide at a magnification of 400×.

### 2.8. Measurement of Cd

The determination of Cd in biological samples was performed by graphite furnace atomic absorption spectrometry (GFAAS) using the stabilized-temperature-platform-furnace coupled with a $D_2$-lamp background correction system. The external quality-control program did not show any time trend in the accuracy of

**Table 1**
Primers, the number of cycles and annealing temperature for RT-PCR.

| Name | Sequence | Denaturation (°C) | Annealing (°C) | Extension (°C) | Number of cycles (*n*) | Size (bp) |
|---|---|---|---|---|---|---|
| GAPDH | Forward: 5′-GAGGGGCCATCCACAGTCTTC-3′<br>Reverse: 5′-CATCACCATCTTCCAGGAGCG-3′ | 94 | 56 °C | 72 | 39 | 340 |
| StAR | Forward: 5′-TGTCAAGGAGATCAAGGTCCTG-3′<br>Reverse: 5′-CGATAGGACCTGGTTGATGAT-3′ | 94 | 57 °C | 72 | 36 | 310 |
| P450scc | Forward: 5′-AGGTGTAGCTCAGGACTTCA-3′<br>Reverse: 5′-AGGAGGCTATAAAGGACACC-3′ | 94 | 56 °C | 72 | 38 | 370 |
| P450 17α | Forward: 5′-CCAGGACCCAAGTGTGTTCT-3′<br>Reverse: 5′-CCTGATACGAAGCACTTCTCG-3′ | 94 | 56 °C | 72 | 37 | 250 |
| 17β-HSD | Forward: 5′-ATTTTACCAGAGAAGACATCT-3′<br>Reverse: 5′-GGGGTCAGCACCTGAATAATG-3′ | 94 | 52 °C | 72 | 39 | 367 |

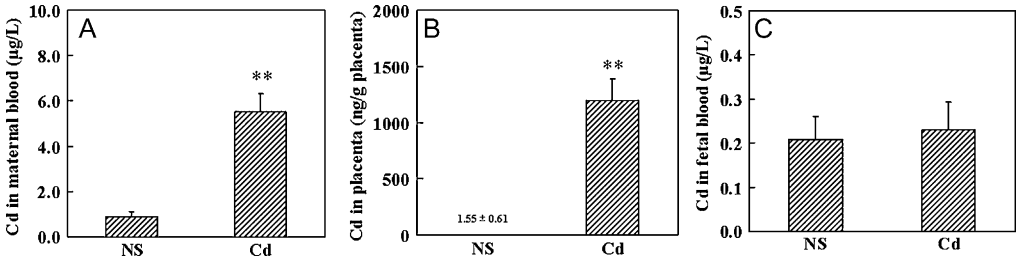


**Fig. 1.** Effects of maternal Cd exposure during late pregnant period on Cd concentration of maternal blood, placenta and fetal blood. Pregnant mice were injected with $CdCl_2$ (0.5 mg/kg) daily from gd 13 to gd 17. NS-treated pregnant mice served as controls. Maternal blood, placenta and fetal blood were collected at gd 18. Cd concentration was measured using GFAAS. (A) Cd concentration in maternal blood; (B) Cd concentration in placenta; (C) Cd concentration in fetal blood. Data were expressed as mean ± SEM of 12 samples from 12 different litters. $^{**}P < 0.01$ significantly different as compared with NS group.

the Cd measurements. Detection limits were based on three times the relative standard deviation of 20 consecutive measurements of the blank solution (1% $HNO_3$) according to IUPAC, being 0.10 μg/L Cd. Precision of the method was measured by coefficients of variation (CV). Mean CV for measurement of Cd in biological samples was 3.5% for within-day determinations and 3.9% for day-to-day determinations. For blood samples, initially studied a 1 + 4 (v/v) dilution with 5% $HNO_3$. For other biological samples (testis, kidney, placenta and liver), samples were decomposed in duplicate according to the following procedure: 200 mg of samples were accurately weighed in a digestion tube, a 3 ml freshly prepared mixture of concentrated $HNO_3$–$H_2O_2$ was added to each tube, and the solutions were kept at room temperature for 12 h, the clear transparent digests were obtained. After this period, the mixture was boiled nearly to dryness, and the residue quantitatively transferred to a 10 ml volumetric flask with 1.0% $HNO_3$. The resulting solution was analyzed by GFAAS by taking 10 μl of the digest under the aforementioned condition.

### 2.9. Statistical analysis

For RT-PCR, StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD mRNA level was normalized to GAPDH mRNA level in the same samples. StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD mRNA level of the control was assigned as 100%. For immunoblotting, StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD were normalized to β-actin level in the same samples. The densitometry unite of the control was assigned as 1. For fetal weight, crown-rump lengths, testis weight, kidney weight, Cd in fetal blood, T in fetal sera, Cd and T analyses in adult offspring, the means were calculated per litter and then averaged per group. All quantified data were expressed as means ± SEM. Student *t* test were used to determine differences between the treated-mice and controls.

## 3. Results

### 3.1. Effects of maternal Cd exposure during late pregnant period on Cd concentration of maternal blood, placenta and fetal blood

To investigate the effects of maternal Cd exposure during the late pregnant period on testicular development of male fetuses, pregnant mice were injected with $CdCl_2$ (0.5 mg/kg) daily from gd 13 to gd 17. As shown in Fig. 1A, Cd concentration in maternal blood was significantly increased in Cd-treated mice. Interestingly, placental Cd level was increased about 750 folds in Cd-treated mice as compared with controls (Fig. 1B). The effects of maternal Cd exposure during the late pregnant period on Cd concentration of fetal blood were analyzed. As shown in Fig. 1C, no significant difference on Cd concentration of fetal blood was observed between Cd-treated mice and controls.

### 3.2. Effects of maternal Cd exposure during late pregnant period on fetal development

No signs of maternal toxicity were observed in dams treated with 0.5 mg/kg $CdCl_2$. No significant difference on body weight gain of dams was observed between Cd-treated pregnant mice and controls (data not shown). Although no significant difference on the number of male live fetuses was observed between Cd-treated mice and controls, the number of female live fetuses was significantly decreased in Cd-treated mice (Table 2). The effects of maternal Cd exposure during the late pregnant period on fetal weight and crown length were analyzed. Both male and female fetal weights were significantly decreased in mice whose mother were exposed to Cd

**Table 2**
Fetal outcomes, fetal weight, crown length, testis and kidney weights.

| | NS | Cd |
|---|---|---|
| No. of litters | 12 | 12 |
| Resoptions per litter | 0.6 ± 0.27 | 0.6 ± 0.44 |
| Dead fetuses per litter | 0.1 ± 0.10 | 0.8 ± 0.43* |
| Live fetuses per litter | 12.2 ± 0.49 | 9.8 ± 1.20* |
| Males | 5.9 ± 0.60 | 5.6 ± 0.84 |
| Females | 6.3 ± 0.54 | 4.2 ± 0.57* |
| Fetal weight (g) | 1.32 ± 0.024 | 1.23 ± 0.027** |
| Males | 1.34 ± 0.022 | 1.26 ± 0.032** |
| Females | 1.30 ± 0.026 | 1.19 ± 0.028** |
| Fetal crown length (mm) | 23.5 ± 0.14 | 22.5 ± 0.18** |
| Males | 23.5 ± 0.22 | 22.5 ± 0.30** |
| Females | 23.5 ± 0.18 | 22.6 ± 0.22** |
| Absolute testis weight (mg) | 0.38 ± 0.022 | 0.26 ± 0.022** |
| Relative testis weight (%) | 0.028 ± 0.005 | 0.021 ± 0.002** |
| Absolute kidney weight (mg) | 4.40 ± 0.198 | 3.49 ± 0.135** |
| Relative kidney weight (%) | 0.328 ± 0.015 | 0.277 ± 0.011** |

* $P<0.05$ as compared with NS group.
** $P<0.01$ as compared with NS group.

during the late pregnant period. In addition, maternal Cd exposure during the late pregnant period significantly reduced fetal crown length (Table 2). The effects of maternal Cd exposure during the late pregnant period on kidney development of fetuses are presented in Table 2. As expected, maternal Cd exposure during the late pregnant period significantly reduced the mean kidney weight of fetuses.

### 3.3. Effects of maternal Cd exposure during late pregnant period on testicular histology and apoptosis

The effects of maternal Cd exposure during the late pregnant period on testicular development of male fetuses were analyzed. As shown in Table 2, the mean testis weight of fetuses was significantly decreased in Cd-treated mice. The effects of maternal Cd exposure during the late pregnant period on histology for fetal testes are presented in Fig. 2B. No abnormal morphology was observed in fetal testes of Cd-treated mice. In addition, no abnormal Leydig cell aggregation was observed in testis of fetuses whose mothers were exposed to Cd during the late pregnant period (Fig. 2D). The effects of maternal Cd exposure during the late pregnant period on apoptosis in fetal testes were determined using TUNEL assay. No TUNEL+ cell was observed in testes of fetuses whose mothers were exposed to Cd during the late pregnant period (Fig. 2F).

### 3.4. Effects of maternal Cd exposure during late pregnant period on serum T in male fetuses

The effects of maternal Cd exposure during the late pregnant period on serum T in male fetuses were analyzed. As shown in Fig. 2G, maternal Cd exposure during the late pregnant period significantly reduced the level of serum T in male fetuses ($1.37 \pm 0.22$ ng/ml vs. $0.69 \pm 0.09$ ng/ml, $P < 0.05$).

### 3.5. Effects of maternal Cd exposure during late pregnant period on the expression of testicular StAR and T biosynthetic enzymes in male fetuses

The effects of maternal Cd exposure during the late pregnant period on mRNA level of StAR and T biosynthetic enzymes in fetal testes were then measured. As expected, mRNA levels of testicular StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD were significantly decreased in fetuses whose mothers were exposed to Cd during the late pregnant period (Fig. 3A, C, E and G). The effects of maternal Cd exposure during the late pregnant period on protein expression of testicular StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD were analyzed. As shown in Fig. 3B, D, F and H, maternal Cd exposure during the late pregnant period reduced protein expression of testicular StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD in fetuses.

### 3.6. Effects of maternal Cd exposure during late pregnant period on Cd concentration of blood, liver, kidney and testis of adult male offspring

As shown in Fig. 4A–C, no significant increase on Cd concentration of blood, liver and kidney of adult male offspring whose mothers were exposed to Cd during the late pregnant period. In addition, maternal Cd exposure during the late pregnant period did not increased testicular Cd level of adult male offspring (Fig. 4D).

### 3.7. Effects of maternal Cd exposure during late pregnant period on weights of male reproductive organs and sperm quality at adulthood

As shown in Table 3, no significant difference on the weight of testes and epididymides at adulthood was observed between Cd-exposed mice and controls. The effects of maternal Cd exposure during the late pregnant period on sperm quality of adult male offspring were analyzed. As shown in Table 3, no significant difference on the number of spermatozoa in cauda epididymides was observed between control mice and mice whose mothers were exposed to Cd during the late pregnant period.

**Table 3**
Body weight, reproductive organs weights and sperm count in adult male offspring.

| | NS | Cd |
|---|---|---|
| Body weight (g) | 35.5 ± 0.43 | 35.5 ± 0.66 |
| Absolute testis weight (mg) | 245.8 ± 5.96 | 257.7 ± 6.32 |
| Relative testis weight (%) | 0.70 ± 0.017 | 0.72 ± 0.019 |
| Absolute epididymides weight (mg) | 91.2 ± 2.48 | 86.7 ± 4.46 |
| Relative epididymides weight (%) | 0.26 ± 0.007 | 0.25 ± 0.013 |
| Sperm count ($\times 10^6$) | 17.4 ± 0.74 | 17.6 ± 1.05 |

### 3.8. Effects of maternal Cd exposure during late pregnant period on histology and apoptotic cells in testes of adult male offspring

The effects of maternal Cd exposure during the late pregnant period on histology in testes of adult offspring were analyzed. As shown in Fig. 5B, no abnormal morphology was observed in testes of adult offspring whose mothers were exposed to Cd during the late pregnant period. However, maternal Cd exposure slightly reduced the number of Leydig cells in testes of adult male offspring. The effects of maternal Cd exposure during the late pregnant period on testicular apoptosis were analyzed in adult male offspring. As shown in Fig. 5F and H, maternal Cd exposure during the late pregnant period did not increase the number of apoptotic cells in testes of adult male offspring.

### 3.9. Effects of maternal Cd exposure during late pregnant period on serum and testicular T in adult male offspring

The effects of maternal Cd exposure during the late pregnant period on serum and testicular T in adult male offspring were analyzed. As shown in Fig. 5I, maternal Cd exposure during the late pregnant period significantly reduced the level of serum T in adult male offspring. In addition, maternal Cd exposure during the late pregnant period significantly reduced the level of testicular T in adult male offspring (Fig. 5J).

### 3.10. Effects of maternal Cd exposure during late pregnant period on the expression of testicular StAR and T biosynthetic enzymes in adult offspring

The effects of maternal Cd exposure during the late pregnant period on mRNA level of testicular StAR and T biosynthetic enzymes were then measured in adult offspring. As shown in Fig. 6A and C, mRNA levels of testicular StAR and P450scc were significantly decreased in Cd-exposed mice. However, maternal Cd exposure during the late pregnant period had little effect on mRNA level of testicular $P450_{17\alpha}$ and 17β-HSD in adult offspring (Fig. 6E and G). The effects of maternal Cd exposure during the late pregnant period on protein expression of testicular StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD were analyzed in adult offspring. As shown in Fig. 6D, protein level of testicular P450scc was significantly decreased in Cd-exposed mice. However, maternal Cd exposure during the late pregnant period had little effect on protein expression of testicular StAR, $P450_{17\alpha}$ and 17β-HSD in adult offspring (Fig. 6B, F and H).

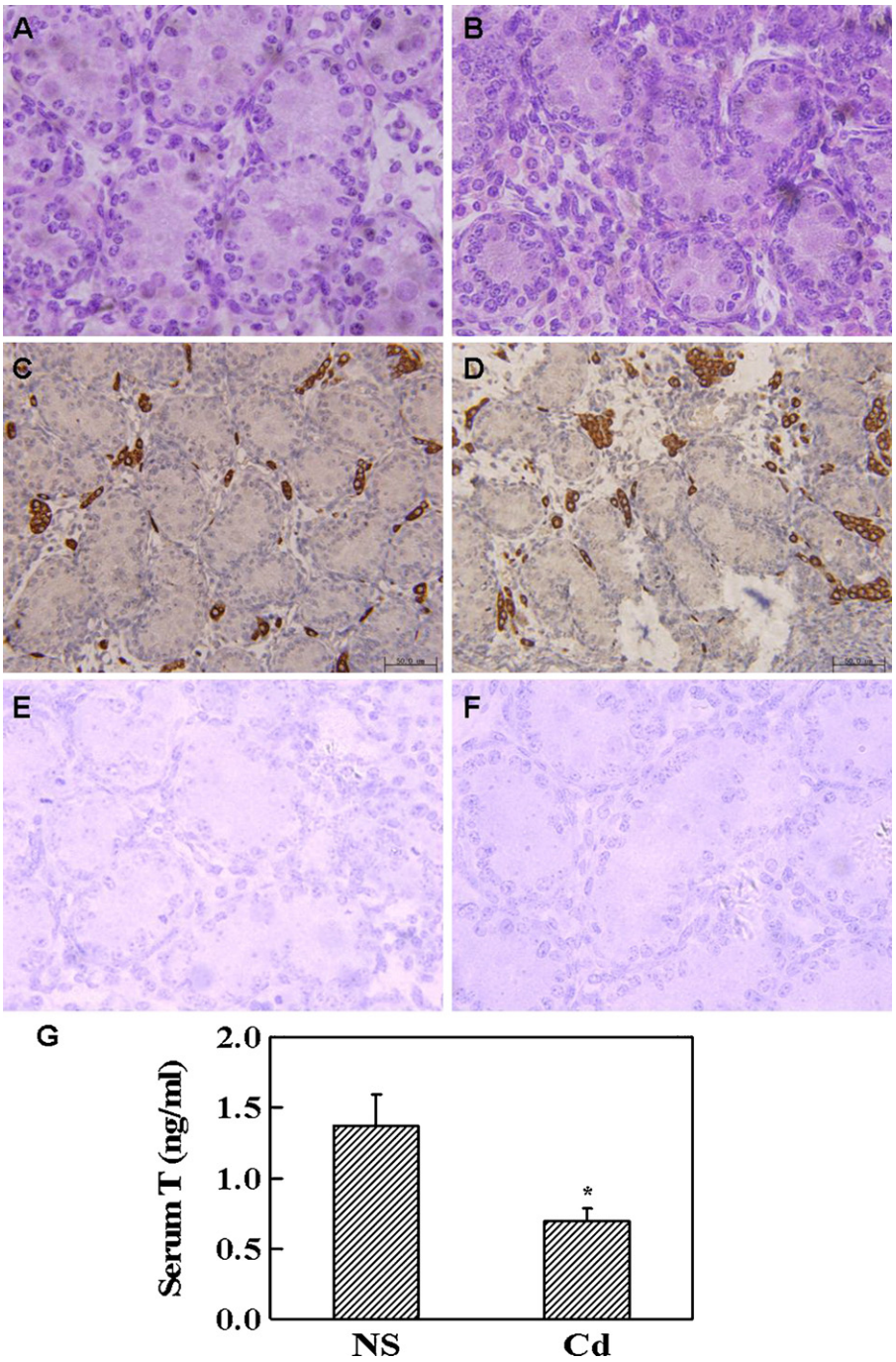


**Fig. 2.** Effects of maternal Cd exposure during late pregnant period on testicular histology and serum T in male fetuses. Pregnant mice were injected with $CdCl_2$ (0.5 mg/kg) daily from gd 13 to gd 17. NS-treated pregnant mice served as controls. Fetal sera and testes were collected at gd 18. Testicular cross-sections from control (A) and Cd-treated (B) mice were stained with H&E. Original magnification: 640×. Leydig cells in fetal testes from control (C) and Cd-treated (D) mice were immunolocalized in the interstitium by staining with a polyclonal antibody specific for mouse 3β-HSD. Original magnification: 400×. Apoptosis was measured with TUNEL staining of testicular sections from control (E) and Cd-treated (F) mice. Original magnification: 640×. (G) Serum T was measured using RIA. Data were expressed as mean ± SEM of 12 samples from 12 different litters. $^*P < 0.05$ significantly different as compared with NS group.

### *3.11. Effects of maternal Cd exposure during late pregnant period on the fertility of adult male offspring*

Fertility analysis showed that all 12 males bred with control females mated and vaginal plugs were found in all females. All females bred with males whose mothers were exposed to Cd during the late pregnant period were found to be fertilized and complete the pregnancy. Surprisingly, the number of live fetuses per litter in F2 generation was significantly decreased in Cd-treated group, whereas there was no significant difference on the numbers of implantation sites per litter and dead fetuses between Cd-treated mice and controls (Table 4). No significant difference on male/female sex ratios and body weight and crown length in F2 generation was observed between Cd-treated mice and controls (Table 4).

## 4. Discussion

In the present study, we first investigated the effects of maternal Cd exposure during the late pregnant period on fetal development.

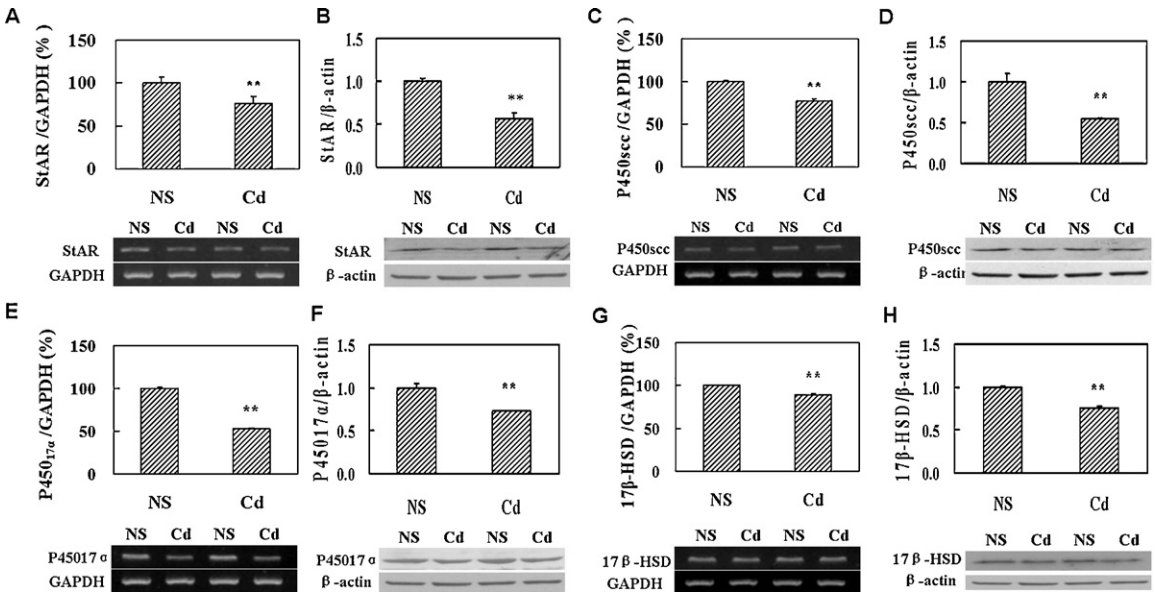


**Fig. 3.** Effects of maternal Cd exposure during late pregnant period on the expression of StAR and T biosynthetic enzymes in fetal testes. Pregnant mice were injected with $CdCl_2$ (0.5 mg/kg) daily from gd 13 to gd 17. NS-treated pregnant mice served as controls. Fetal testes were collected at gd 18. StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD mRNA was measured using RT-PCR. (A) StAR, (C) P450scc, (E) $P450_{17\alpha}$ and (G) 17β-HSD mRNA were normalized to GAPDH mRNA level in the same samples. StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD mRNA level of the control was assigned as 100%. The protein expression of StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD was measured using immunoblotting. (B) StAR, (D) P450scc, (F) $P450_{17\alpha}$ and (H) 17β-HSD were normalized to β-actin level in the same samples. The densitometry unite of the control was assigned as 1. All data were expressed as means ± SEM of six samples from six different litters. $^{**}P < 0.01$ significantly different as compared with NS group.

We found that both male and female fetal weights were significantly decreased in mice whose mothers were exposed to Cd during the late pregnant period. In addition, maternal Cd exposure during the late pregnant period significantly reduced fetal crown length. Importantly, absolute and relative weights of testes were significantly decreased in male fetuses whose mothers were exposed to a low dose of Cd during the late pregnant period. In addition, the level of serum T was significantly decreased in male fetuses whose mothers were exposed to Cd during the late pregnant period. These results suggest that maternal Cd exposure during the late pregnant period could reduce testis weight and impair testicular steroidogenesis in fetal period.

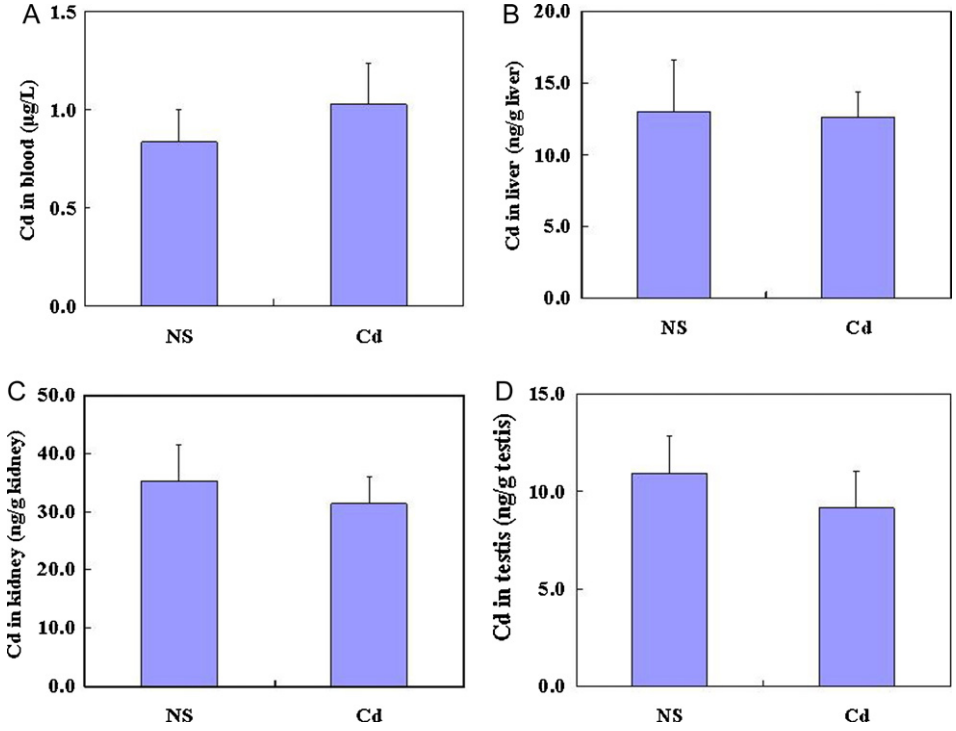


**Fig. 4.** Effects of maternal Cd exposure during late pregnant period on Cd concentration in blood, liver, kidney and testis of adult male offspring. Pregnant mice were injected with $CdCl_2$ (0.5 mg/kg) daily from gd 13 to gd 17. NS-treated pregnant mice served as controls. Blood, liver, kidney and testis of adult male offspring were collected at PND 70. Cd measured using GFAAS. (A) Cd concentration in blood; (B) Cd concentration in liver; (C) Cd concentration in kidney; (D) Cd concentration in testis. All data were expressed as means ± SEM of 12 samples.

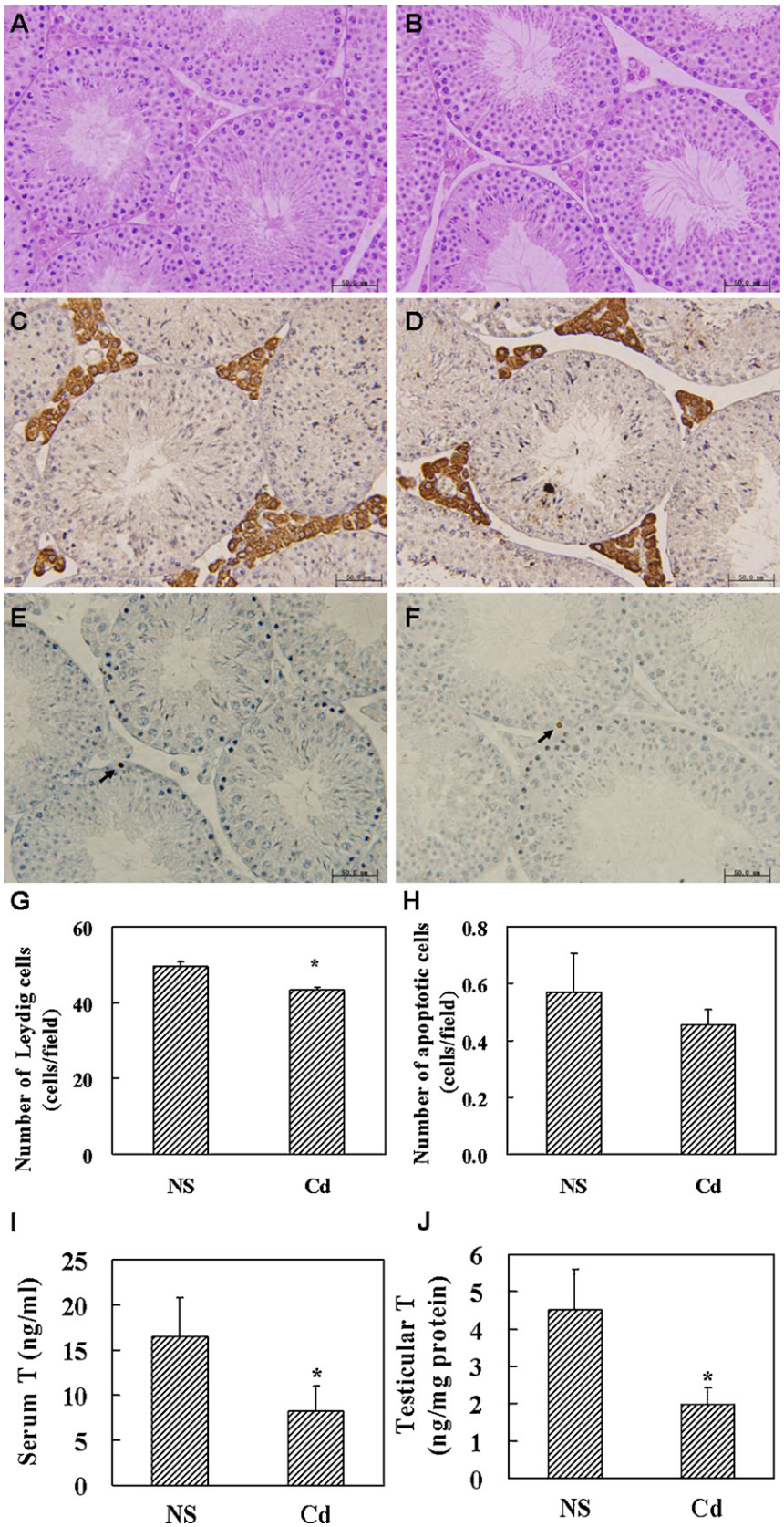


**Fig. 5.** Effects of maternal Cd exposure during late pregnant period on testicular histology and T in serum and testis of adult male offspring. Pregnant mice were injected with $CdCl_2$ (0.5 mg/kg) daily from gd 13 to gd 17. NS-treated pregnant mice served as controls. Sera and testes were collected at PND70. Testicular cross sections from (A) control and (B) Cd-treated mice were stained with H&E. Leydig cells in testes from (C) control and (D) Cd-treated mice were immunolocalized by staining with a polyclonal antibody specific for mouse 3β-HSD. Apoptosis was analyzed with TUNEL staining of testicular sections from (E) control and (F) Cd-treated mice. (G) The number of Leydig cells in testes was counted in 12 randomly selected fields from each slide at 400× magnification. (H) The number of TUNEL+ cells was counted in 12 randomly selected fields from each slide at 400× magnification. (I) Serum and (J) testicular T was measured using RIA. All data were expressed as means ± SEM of 12 samples from six different litters. $^{*}P < 0.05$ as compared with NS group.

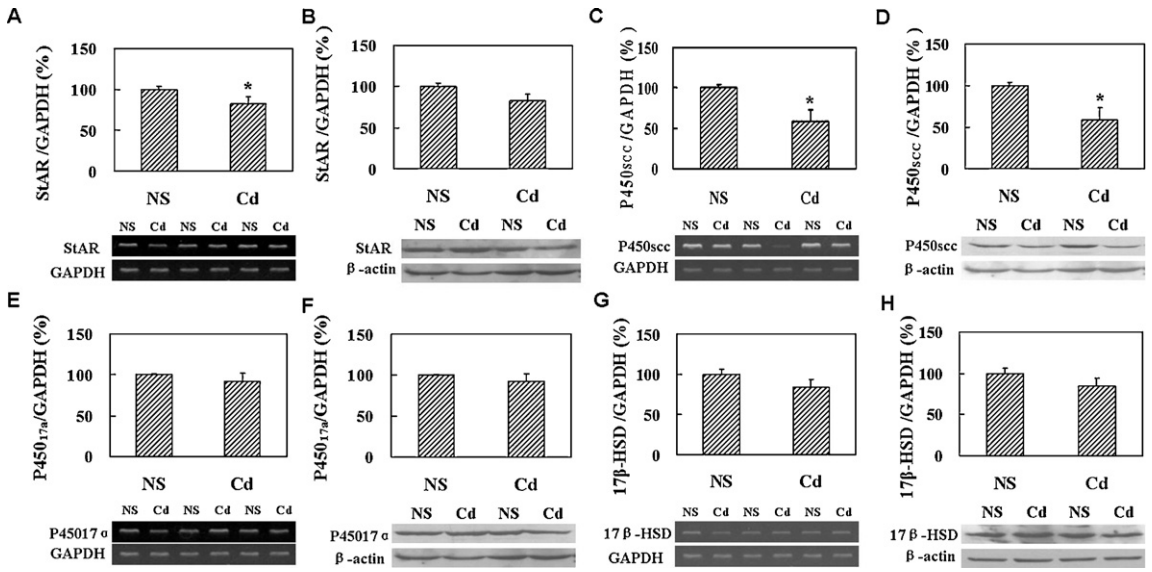


**Fig. 6.** Effects of maternal Cd exposure during late pregnant period on the expression of StAR and T biosynthetic enzymes in testes of adult offspring. Pregnant mice were injected with $CdCl_2$ (0.5 mg/kg) daily from gd 13 to gd 17. NS-treated pregnant mice served as controls. Testes were collected at PND70. StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD mRNA was measured using RT-PCR. (A) StAR, (C) P450scc, (E) $P450_{17\alpha}$ and (G) 17β-HSD mRNA was normalized to GAPDH mRNA level in the same samples. StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD mRNA level of the control was assigned as 100%. The protein expression of StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD was measured using immunoblotting. (B) StAR, (D) P450scc, (F) $P450_{17\alpha}$ and (H) 17β-HSD was normalized to β-actin level in the same samples. The densitometry unite of the control was assigned as 1. All data were expressed as means ± SEM of six samples from six different litters. *$P < 0.05$ significantly different as compared with NS group.

StAR is an essential and limiting factor in testicular T synthesis, responsible for the transport of cholesterol into mitochondria (Miller, 2007). A recent study demonstrated that adult Cd exposure obviously reduced protein level of StAR in Leydig cells of rats (Gunnarsson et al., 2004). Our recent study showed that pubertal Cd exposure (1.0 mg/kg) inhibited StAR expression in testes (Ji et al., 2010). In the present study, we found that mRNA level of StAR in fetal testes was significantly decreased in mice whose mothers were exposed to a low dose Cd (0.5 mg/kg) during the late pregnant period. In addition, maternal Cd exposure during the late pregnant period markedly reduced protein expression of StAR in fetal testes. Interestingly, the present study showed that the expression of P450scc, $P450_{17\alpha}$ and 17β-HSD, three key enzymes for T synthesis (Payne and Youngblood, 1995), were obviously down-regulated in testes of fetuses whose mothers were exposed to Cd during the late pregnant period. These results suggest that the decreased T syntheses might, at least partially, be attributed to the decreased expression of testicular StAR and T synthetic enzymes.

How Cd impairs testicular steroidogenesis in fetuses remains obscure. The present study showed that only traces of blood Cd was measured in fetuses whose mothers were exposed to Cd during the late pregnant period. Importantly, no significant elevation of blood Cd concentration was observed in fetuses whose mothers were exposed to Cd during the late pregnant period. These results are in agreement with others (Whelton et al., 1993; Brako et al., 2003), in which only less than 0.1% of Cd was passed from dams to fetuses when pregnant mice were chronically exposed to tracer levels of $^{109}Cd$ in drinking water. These results suggest that placenta could deter most of Cd from passing from dams to fetuses. Thus, the impairments on testicular steroidogenesis in fetuses, caused by maternal Cd exposure during the late pregnant period, can not be completely attribute to Cd-induced direct toxic effect on fetuses. Indeed, Cd could accumulate in placenta (Boadi et al., 1991). The present study showed that placental Cd level was increased about 750 folds in pregnant mice injected with Cd. Increasing evidence demonstrated that the accumulation of Cd in placenta impaired the transport of micronutrients, such as zinc, to the fetuses (Kuriwaki et al., 2005; Kippler et al., 2010). Several earlier studies found that maternal Cd exposure during pregnancy resulted in a significant reduction of zinc concentration in fetal and neonatal liver (Hazelhoff Roelfzema et al., 1988, 1989). Zinc is an important trace element for fetal development. Several studies showed that zinc deficiency delayed fetal growth (Shahbazi et al., 2009). An earlier study showed that zinc deficiency impairs testicular development and steroidogenesis (Hamdi et al., 1997). Interestingly, zinc supplement abolished not only Cd-induced reduction of T production but also the downregulation of StAR in testes of adult rats (Gunnarsson et al., 2004). Thus, we speculate that Cd-induced impediment in the transport of micronutrients mainly zinc from dams to fetuses might contribute, at least partially, to the impairment on testicular steroidogenesis in fetuses. Additional study is required to determine whether zinc supplement could alleviate Cd-induced impairment on testicular steroidogenesis in fetuses.

Several studies showed that administration to adult rodents with high doses of $CdCl_2$ (5–20 μmol/kg) resulted in germ cell apoptosis in testes (Xu et al., 1996; Zhou et al., 1999; Ozawa et al., 2002). Recently, we found that pubertal exposure to 1.0 mg/kg Cd daily for five weeks markedly increased the number of apoptotic cells in testes of adult mice (Ji et al., 2010). In the present study, we investigated the effects of maternal Cd exposure during the late

**Table 4**
Fetal outcomes, fetal weight and crown length in F2 generation.

| | NS | Cd |
|---|---|---|
| No. of litter | 12 | 12 |
| Resoptions per litter | 0.15 ± 0.15 | 1.00 ± 0.42* |
| Dead fetuses per litter | 0.0 ± 0.00 | 0.4 ± 0.26 |
| Implantation sites per litter | 13.7 ± 0.87 | 12.6 ± 0.65 |
| Live fetuses per litter | 13.4 ± 0.81 | 11.3 ± 0.31* |
| Males | 6.4 ± 0.61 | 4.9 ± 0.61 |
| Females | 7.0 ± 1.00 | 6.4 ± 0.68 |
| Fetal weight (g) | 1.36 ± 0.029 | 1.41 ± 0.036 |
| Males | 1.39 ± 0.026 | 1.33 ± 0.035 |
| Females | 1.43 ± 0.042 | 1.41 ± 0.033 |
| Fetal crown length (mm) | 25.2 ± 0.04 | 24.8 ± 0.04 |
| Males | 25.0 ± 0.36 | 25.1 ± 0.05 |
| Females | 25.4 ± 0.08 | 24.5 ± 0.04 |

Data were expressed as means ± SEM.
* $P < 0.05$ as compared with NS group.

pregnant period on germ cell apoptosis in fetal testes. Interestingly, no TUNEL+ germ cell was observed in testes of fetuses whose mothers were exposed to low dose Cd (0.5 mg/kg) daily during the late pregnant period. Moreover, maternal Cd exposure during the late pregnant period did not result in abnormal Leydig cell aggregation in testes of male fetuses. In addition, no abnormal morphology was observed in fetal testes of Cd-treated mice. These results suggest that maternal exposure to low dose Cd during the late pregnant period does not cause an obvious pathological impairment on fetal testes.

In the present study, we investigated the effects of maternal Cd exposure during the late pregnant period on steroidogenesis and spermatogenesis of adult male offspring. We found that maternal Cd exposure during the late pregnant period had no effect on the weight of testes and epididymides and sperm count of adult male offspring. Moreover, no obvious pathological injury was observed in testes of adult male pups whose mothers were exposed to Cd during the late pregnant period. In addition, maternal Cd exposure during the late pregnant period did not increase the number of apoptotic cells in testes of adult male offspring. These results provide further evidences that the reduction of testicular weight in fetal period, caused by maternal Cd exposure during the late pregnant period, is reversible. However, the present study found that maternal Cd exposure during the late pregnant period significantly reduced the level of serum and testicular T in adult male offspring. In addition, the expression of testicular P450scc was significantly decreased in adult male offspring of Cd-exposed mice. Thus, the impairment of maternal Cd exposure during the late pregnant period on testicular steroidogenesis was irreversible.

In summary, the present study indicates that maternal Cd exposure during the late pregnant period reduces testis weight in fetal period. In addition, maternal Cd exposure during the late pregnant period down-regulates the expression of StAR and several key enzymes for T syntheses and inhibited testicular T production in male offspring. Importantly, placenta could deter most of Cd from passing from dams to fetuses. Thus, the impairments on testicular steroidogenesis in fetuses, caused by maternal Cd exposure during the late pregnant period, could not be attributed to a direct action of Cd on fetal testes.

## Conflict of interest

There are no conflicts of interest.

## Acknowledgements

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## References

Angenard, G., Muczynski, V., Coffigny, H., Pairault, C., Duquenne, C., Frydman, R., Habert, R., Rouiller-Fabre, V., Livera, G., 2010. Cadmium increases human fetal germ cell apoptosis. Environ. Health Perspect. 118, 331–337.

Barr Jr., M., 1973. The teratogenicity of cadmium chloride in two stocks of Wistar rats. Teratology 7, 237–242.

Beveridge, R., Pintos, J., Parent, M.E., Asselin, J., Siemiatycki, J., 2010. Lung cancer risk associated with occupational exposure to nickel, chromium VI, and cadmium in two population-based case-control studies in Montreal. Am. J. Ind. Med. 53, 476–485.

Boadi, W.Y., Yannai, S., Urbach, J., Brandes, J.M., Summer, K.H., 1991. Transfer and accumulation of cadmium, and the level of metallothionein in perfused human placentae. Arch. Toxicol. 65, 318–323.

Brako, E.E., Wilson, A.K., Jonah, M.M., Blum, C.A., Cerny, E.A., Williams, K.L., Bhattacharyya, M.H., 2003. Cadmium pathways during gestation and lactation in control versus metallothoinein 1,2-knockout mice. Toxicol. Sci. 71, 154–163.

Gunnarsson, D., Svensson, M., Selstam, G., Nordberg, G., 2004. Pronounced induction of testicular PGF(2 alpha) and suppression of testosterone by cadmium-prevention by zinc. Toxicology 200, 49–58.

Hamdi, S.A., Nassif, O.I., Ardawi, M.S., 1997. Effect of marginal or severe dietary zinc deficiency on testicular development and functions of the rat. Arch. Androl. 38, 243–253.

Hazelhoff Roelfzema, W., Roelofsen, A.M., Leene, W., Peereboom-Stegeman, H.J., 1989. Effects of cadmium exposure during pregnancy on cadmium and zinc concentrations in neonatal liver and consequences for the offspring. Arch. Toxicol. 63, 38–42.

Hazelhoff Roelfzema, W., Roelofsen, A.M., Herber, R.F., Peereboom-Steg, J.H., 1988. Cadmium and zinc concentrations in fetal and maternal rat tissue after parenteral administration of cadmium during pregnancy. Arch. Toxicol. 62, 285–290.

Honda, R., Swaddiwudhipong, W., Nishijo, M., Mahasakpan, P., Teeyakasem, W., Ruangyuttikarn, W., Satarug, S., Padungtod, C., Nakagawa, H., 2010. Cadmium induced renal dysfunction among residents of rice farming area downstream from a zinc-mineralized belt in Thailand. Toxicol. Lett. 198, 26–32.

Hovland Jr., D.N., Machado, A.F., Scott Jr., W.J., Collins, M.D., 1999. Differential sensitivity of the SWV and C57BL/6 mouse strains to the teratogenic action of single administrations of cadmium given throughout the period of anterior neuropore closure. Teratology 60, 13–21.

Ji, Y.L., Wang, H., Liu, P., Wang, Q., Zhao, X.F., Meng, X.H., Yu, T., Zhang, H., Zhang, C., Zhang, Y., Xu, D.X., 2010. Pubertal cadmium exposure impairs testicular development and spermatogenesis via disrupting testicular testosterone synthesis in adult mice. Reprod. Toxicol. 29, 176–183.

Joseph, P., 2009. Mechanisms of cadmium carcinogenesis. Toxicol. Appl. Pharmacol. 238, 272–279.

Kim, J., Soh, J., 2009. Cadmium-induced apoptosis is mediated by the translocation of AIF to the nucleus in rat testes. Toxicol. Lett. 188, 45–51.

Kippler, M., Hoque, A.M., Raqib, R., Ohrvik, H., Ekström, E.C., Vahter, M., 2010. Accumulation of cadmium in human placenta interacts with the transport of micronutrients to the fetus. Toxicol. Lett. 192, 162–168.

Kuriwaki, J., Nishijo, M., Honda, R., Tawara, K., Nakagawa, H., Hori, E., Nishijo, H., 2005. Effects of cadmium exposure during pregnancy on trace elements in fetal rat liver and kidney. Toxicol. Lett. 156, 369–376.

Lafuente, A., Cano, P., Esquifino, A., 2003. Are cadmium effects on plasma gonadotropins, prolactin, ACTH, GH and TSH levels, dose-dependent? Biometals 16, 243–250.

Lafuente, A., González-Carracedo, A., Romero, A., Cano, P., Esquifino, A.I., 2004. Cadmium exposure differentially modifies the circadian patterns of norepinephrine at the median eminence and plasma LH, FSH and testosterone levels. Toxicol. Lett. 146, 175–182.

Laskey, J.W., Phelps, P.V., 1991. Effect of cadmium and other metal cations on in vitro Leydig cell testosterone production. Toxicol. Appl. Pharmacol. 108, 296–306.

Miller, W.L., 2007. StAR search—what we know about how the steroidogenic acute regulatory protein mediates mitochondrial cholesterol import. Mol. Endocrinol. 21, 589–601.

Ozawa, N., Goda, N., Makino, N., Yamaguchi, T., Yoshimura, Y., Suematsu, M., 2002. Leydig cell-derived heme oxygenase-1 regulates apoptosis of premeiotic germ cells in response to stress. J. Clin. Invest. 109, 457–467.

Paniagua-Castro, N., Escalona-Cardoso, G., Chamorro-Cevallos, G., 2007. Glycine reduces cadmium-induced teratogenic damage in mice. Reprod. Toxicol. 23, 92–97.

Pant, N., Upadhyay, G., Pandey, S., Mathur, N., Saxena, D.K., Srivastava, S.P., 2003. Lead and cadmium concentration in the seminal plasma of men in the general population: correlation with sperm quality. Reprod. Toxicol. 17, 447–450.

Payne, A.H., Youngblood, G.L., 1995. Regulation of expression of steroidogenic enzymes in Leydig cells. Biol. Reprod. 52, 217–225.

Robinson, J.F., Yu, X., Hong, S., Griffith, W.C., Beyer, R., Kim, E., Faustman, E.M., 2009. Cadmium-induced differential toxicogenomic response in resistant and sensitive mouse strains undergoing neurulation. Toxicol. Sci. 107, 206–219.

Ronco, A.M., Montenegro, M., Castillo, P., Urrutia, M., Saez, D., Hirsch, S., Zepeda, R., Llanos, M.N., 2011. Maternal exposure to cadmium during gestation perturbs the vascular system of the adult rat offspring. Toxicol. Appl. Pharmacol. 251, 137–145.

Scott Jr., W.J., Schreiner, C.M., Goetz, J.A., Robbins, D., Bell, S.M., 2005. Cadmium-induced postaxial forelimb ectrodactyly: association with altered sonic hedgehog signaling. Reprod. Toxicol. 19, 479–485.

Sen Gupta, R., Kim, J., Gomes, C., Oh, S., Park, J., Im, W.B., Seong, J.Y., Ahn, R.S., Kwon, H.B., Soh, J., 2004. Effect of ascorbic acid supplementation on testicular steroidogenesis and germ cell death in cadmium-treated male rats. Mol. Cell. Endocrinol. 221, 57–66.

Shahbazi, M., Naghdi, N., Tahmasebi, S., Sheikh, M., Namvar, Asl, N., Kazemnejad, A., 2009. The effect of iron and zinc dietary restriction of pregnant rats on physical growth of litters. Biol. Trace Elem. Res. 128, 232–238.

Siu, E.R., Mruk, D.D., Porto, C.S., Cheng, C.Y., 2009. Cadmium-induced testicular injury. Toxicol. Appl. Pharmacol. 238, 240–249.

Telisman, S., Cvitković, P., Jurasović, J., Pizent, A., Gavella, M., Rocić, B., 2000. Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc, and copper in men. Environ. Health Perspect. 108, 45–53.

Waalkes, M.P., 2000. Cadmium carcinogenesis in review. J. Inorg. Biochem. 79, 241–244.

Whelton, B.D., Toomey, J.M., Bhattacharyya, M.H., 1993. Cadmium-109 metabolism in mice. IV. Diet versus maternal stores as a source of cadmium transfer to mouse fetuses and pups during gestation and lactation. J. Toxicol. Environ. Health 40, 531–546.

Wu, H.M., Lin-Tan, D.T., Wang, M.L., Huang, H.Y., Wang, H.S., Soong, Y.K., Lin, J.L., 2008. Cadmium level in seminal plasma may affect the pregnancy rate for patients undergoing infertility evaluation and treatment. Reprod. Toxicol. 25, 481–484.

Xu, B., Chia, S.E., Tsakok, M., Ong, C.N., 1993. Trace elements in blood and seminal plasma and their relationship to sperm quality. Reprod. Toxicol. 7, 613–618.

Xu, C., Johnson, J.E., Singh, P.K., Jones, M.M., Yan, H., Carter, C.E., 1996. In vivo studies of cadmium-induced apoptosis in testicular tissue of the rat and its modulation by a chelating agent. Toxicology 107, 1–8.

Xu, D.X., Shen, H.M., Zhu, Q.X., Chua, L.H., Wang, Q.N., Chia, S.E., Ong, C.N., 2003. The associations among semen quality, oxidative DNA damage in human spermatozoa and concentrations of cadmium, lead and selenium in seminal plasma. Mutat. Res. 534, 155–163.

Zhou, T., Zhou, G., Song, W., Eguchi, N., Lu, W., Lundin, E., Jin, T., Nordberg, G., 1999. Cadmium-induced apoptosis and changes in expression of p53, c-jun and MT-I genes in testes and ventral prostate of rats. Toxicology 142, 1–13.