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Effect of Exposure to Cadmium during Pregnancy on Pregnancy Outcomes of Dams and Sexual Development and Fertility In Female Progeny of Wistar Rats

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ABSTRACT

The aim of the present study was to assess the reproductive toxic effects of cadmium in adult female rats exposed during pregnancy and further to assess the reproductive toxicity of female progeny. Pregnant rats were injected intra peritoneal with either 0.5 or 5.0 µg cadmium/kg body weight from days 12 to 19 of gestation. No significant changes in pregnancy duration and litter size and birth weight of pups were observed in rats exposed to cadmium when compared to controls. The viability and weaning indices of pups exposed to cadmium during embryonic development were comparable with the control group. The duration of estrous cycle of female offspring is decreased significantly in rats exposed to cadmium during embryonic development. The mating and fertility indices of female progeny exposed to cadmium during embryonic development were comparable with controls. The mean conception time and mean number of implantations and live fetuses in female progeny mated with control males were also comparable with controls. The data presented in the present study reveal no significant changes in pregnancy output of dams exposed to environmentally relevant amounts of cadmium. However, female offspring experienced and early onset of puberty, decrease in estrous cycle duration without any change in fertility.

KEYWORDS: Cadmium, Gestation, Female progeny, Estrous cycle, Fertility.

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1. INTRODUCTION

Cadmium (Cd) is one of the common toxic heavy metals and is widely distributed in the environment. Cd is listed as 7th hazardous substance by the Agency for Toxic Substances and Disease Registry (ATSDR) and Environmental Protection Agency (EPA) of the United States.[1,2] Diet is the most important source of Cd exposure in the general population (non-occupational and non-smoking).[2] Both natural and anthropogenic sources of this heavy metal, including industrial emissions and the application of fertilizer and sewage sludge to farm land, may lead to the contamination of soils resulting in increased Cd uptake by crops and vegetables grown for human consumption.[3,4] Cadmium is used in industrial activities such as the manufacture of nickel-cadmium batteries, electroplating, pigments, ceramics, plastic stabilizers, and fertilizers, as well as in the mining, and agricultural activities.[5,6,7] Tobacco smoke is another important source of Cd exposure, it causes significant rise in blood cadmium levels in smokers than non-smokers.[8,9,10] The most dangerous characteristic of cadmium is that it accumulates throughout a lifetime due to its non-biodegradable nature and long biological half-life.[11] In addition, it is estimated that dietary intake of cadmium is higher in men than women.[12]

Cd exposure has been associated with a wide range of toxic effects including nephrotoxicity, carcinogenity, teratogenicity, endocrine, and immune toxicities.[13,14,15] Exposure to Cd also results in hepatic damage, renal dysfunction, hypertension, central nervous system injury, testicular atrophy.[16,17,18,19] Cadmium has also been identified as an endocrine disruptor.[20] The toxicological evidence with respect to cadmium's effects on the reproductive system was recently reviewed.[21,22,23] Acute exposure to Cd results in significant decrease progesterone production in female rats, and this effect is dependent on the stage of the estrus cycle at the time of exposure.[24] More recently, it has been reported that Cd also possesses estrogenic properties.[25] Cd has been reported to bind and activate estrogen receptor α (ERα) *in vitro* and *in vivo* thereby mimic the effects of estrogen on the uterus and mammary gland.[25,26]

In rats, exposure to cadmium dusts causes change in duration of the estrous cycle.[27] Cd was known to accumulate in placenta[28] and smoking during pregnancy was shown to increase placental cadmium concentration and effect placental morphology.[29] From the above literature review, it is evident that Cd accumulates in reproductive tissues and affects steroidogenesis thereby reproduction in animals. Though much data are available on Cd toxicity in adult animals, data related to exposure to Cd during pregnancy and progeny reproduction are limited. The present study was designed to determine the health and fertility output of dams exposed to Cd during pregnancy and reproductive aspects of female offspring.

2. MATERIALS AND METHODS

2.1. Chemicals

Cadmium chloride (99.99%) was purchased from Sigma Chemical Company, St Louis, MO, USA. All other chemicals used in study were of analytical grade and obtained from local commercial sources.

2.2. Procurement and maintenance of experimental animals

Wistar strain female rats were purchased from an authorized vendor (M/S Raghavendra enterprises, Bengaluru, India) and used for the present study. Upon arrival, rats were housed in polypropylene cages (18" x 10" x 8") containing sterilized paddy husk as bedding material, and provided filtered tap water and standard rodent feed (purchased from Sai Durga Agencies, Bengaluru, India) *ad libitum*. Animals were maintained in a well-controlled (temperature 22-25°C; 12:12 hr light: dark cycle, humidity 50 ± 5%) animal house facility at Department of Zoology, S.V. University. The growth of the animals was monitored regularly (by determining body weight changes) and the animals displaying poor growth rate were discarded from the experiment. The experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. All the procedures were approved by the Institutional Animal Ethical Committee (Regd. No.438/01/a/CPCSEA/dated 17-07-2001) with a resolution No.10/ (i)/a/CPCSEA/IAEC/SVU/ ZOOL/ PSR/dated 08-07-2012.

2.3. Experimental design

Ninety day old female rats (body weight 240 ± 15 g) with normal estrus cyclic pattern were selected for the present investigation. Female rats in pro-estrous stage were cohabited with ninety day old (body weight 230 ± 15 g) male rats (1:1 ratio). Copulation was examined every morning and confirmed by the presence of a vaginal plug and/or sperm in vaginal washings. The cohabitation period was 5 days. On the day when copulation was confirmed, female rats were moved into separate cages and housed individually. The presence of sperm in the vaginal smear was considered as sperm positive female and day 0 of gestation (GD 0).

Pregnant rats were randomly allocated into three groups. Rats in group 1 served as controls and animals in group 2 and 3 were injected intra peritoneally with either 0.5 or 5.0 µg cadmium/Kg body weight from days 12 to 19 of gestation (GD 12 – 19). Cadmium chloride was dissolved in sterile phosphate buffered saline (PBS) and administered as a single intra-peritoneal injection at a dose of either 0.5 or 5.0 µg/Kg body weight/day. All the animals were allowed to deliver pups. Two

days after birth, the offspring were cross-fostered. Ten pups (5 male and 5 females) were housed with a lactating dam and were weaned on postnatal day 22. Thereafter male and female animals were housed separately in groups of three to five.

2.4. Pregnancy outcomes

Maternal body weight was monitored daily during gestation. Additionally, the weight of the dams on GD 21 was corrected by subtracting the sum of the weights of the newborns on PND 1. Using this calculation, we could better estimate the possible maternal and/or foetal toxicity. During these periods, general clinical observations were made once per day. All of the animals were evaluated for mortality, morbidity and general clinical signs, such as behavioral changes (e.g. agitation, lethargy, hyperactivity and cannibalism), neurological changes (e.g. convulsions, tremors, muscle rigidity and hyper-reflexia) and autonomic signs (e.g. lacrimation, piloerection, pupil size and unusual respiratory pattern). Additionally, the following data were recorded: pregnancy length, litter size, and pup birth weight. The viability index [(number of live offspring on PND 4/number of live offspring delivered) X 100], and the weaning index [(number of live offspring on PND 21/number of live offspring delivered) X 100] were also evaluated.

2.5. Vaginal smear cytology and description of estrous stages of female progeny

From the postnatal day 70, for 20 consecutive days, vaginal smears were prepared every morning (6.00 – 8.00 h) to characterize the estrous cycle. Different stages of estrous cycle were determined using the method described by Zarrow et al.[30] and reviewed by Cooper et al.[31] A small amount of a physiological saline (0.9% NaCl) solution was introduced carefully in the vagina by means of a disposable pasture pipette. The saline solution was sucked in quickly back into the pipette. The smear was prepared on a clean glass slide and examined under microscope for various stages of estrous cycle and also to determine the mating (based on the presence of sperm).

The stages of estrous cycle can be differentiated by characteristic cell types that are visible during each stage and differences in cell density. Pro-estrus smear largely consist of small, round nucleated epithelial cells singly or in sheets. Estrus is the period when the female is amenable (sexually receptive) to the male for mating and vaginal smear becomes “cheesy” with masses of adherent non-nucleated cornified cells. Formation of the corpus luteum (corpora lutea with multiple ovulations) occurs during the met-estrus stage and vaginal smear contains nearly all types of cells such as leucocytes, few epithelial cells and cornified cells. During di-estrus stage vaginal smear becomes stringy, mucus entangled largely with leucocytes and few nucleated epithelial cells. Duration of each estrous cycle (4.4 - 4.7 days) and length of each stage of cycle (pro-estrous (14 - 18

h), estrous (25 - 38 h), met-estrous (5 - 8 h) and di-estrous (53 - 59 h)) varies depending on rat strain.[32]

2.6. Reproductive performance examinations

On PND 100, female rats from control and experimental groups in proestrous stage were cohabited with untreated 100 day old male rats (1:1 ratio) to evaluate their reproductive ability. Male rat was introduced into the home cage of female rat. Successful mating was confirmed by the presence of vaginal plug or sperm in vaginal washing. Pregnant rats were sacrificed on 18th day of gestation; both ovaries were removed and examined for the number of corpora lutea. Uterine horns from both sides were removed and numbers of implantations and live/dead fetuses were counted. Mating index (number of sperm positive females/number of pairings×100), fertility index (number of pregnant females/number of pairings×100), pre-implantation loss (difference between the number of corpora lutea and the number of implantation sites expressed as per number of corpora lutea), and post-implantation loss (difference between the number of implantations and the number of live fetuses expressed as per number of implantations) were calculated. In addition, the conception time, the interval between the first day of cohabitation and the day of vaginal plug and/or sperm in vaginal smear, was recorded for each female.

2.7. Statistical treatment of the data

The data were statistically analyzed using One-way Analysis of Variance (ANOVA) followed by Tukey's test. The data were expressed as mean ± S.D. and 'p' value < 0.05 was considered significant. All statistical tests were performed using Statistical Package for Social Sciences (SPSS Inc., Chertsey, UK).

3. RESULTS AND DISCUSSION

In the present study, all the animals were apparently normal and no unusual behaviors (viz. head flicking, head searching, biting, licking, self-mutilation, circling, and walking back-wards) were observed in any of the pregnant rats. No significant changes in pregnancy duration and litter size were observed in rats exposed to cadmium when compared to controls (Table No. 1).

The body weight gain of dams exposed to either 0.5 or 5.0 mg Cd/kg body weight during pregnancy was comparable with controls (Figure No. 1A). Similarly, the corrected body weight on GD 21 in the dams exposed to Cd was also comparable with the control group (Figure No. 1B). Pregnancy length, litter size, and pup birth weight were unaffected by Cd exposure. The viability and weaning indices were also comparable in dams exposed to Cd compared with the control group (Table No. 1).

In the present study, though the mean length of estrous stage is not significantly different, the duration of estrous cycle is decreased significantly in rats exposed to different doses of cadmium during embryonic development (Table No. 2; Figure No. 2).

An estrous cycle is a rhythmic reproductive cycle occurring in sexually mature female mammals which depend upon the periodic release of gonadotropic releasing hormones, gonadotropins and sex hormones and gives a fair index of ovarian and uterine function. Previous studies reported that, pregnant and lactating female animals were reported to absorb and retain substantially more dietary Cd than do their non-pregnant counterparts and Cd can be transferred to fetus.[33] Previous studies also reported that exposure to heavy metals caused disruption in the duration of estrous cyclicity.[34] Cd is a known endocrine disruptor by affecting the synthesis and/or regulation of several hormones.[35,36] Cd treatment had adverse effects on female reproductive system and pregnancy outcome.[13]

Another reproductive parameter studied was the effect of prenatal cadmium treatment on fertility related parameters in female progeny, is the presence of copulatory plugs or sperm in vaginal washings in experimental females mated with control males. The data suggests that mating and fertility efficiency is not compromised in experimental female offspring. The mean conception time was also comparable in all groups. Female progeny mated with control males had implantations similar to controls. The fetal loss may occur both before and after implantation. The number of implantations and live fetuses in female offspring exposed to Cd during embryonic development mated with control males were comparable with controls (Table No. 3). Previous studies reported that acute and at high-dose administration of cadmium has been shown to affect various female reproductive end points in rats, resulting in hemorrhagic changes in the ovary and uterus resulting in irregular estrous and unbalanced ovulation.[37] Piaseket al.[38] evaluated the *in vivo* effects of cadmium on steroidogenesis in rat ovaries resulting in severe damage to embryos and the reproductive organs in adults including the ovary and testes.

Table No. 1:"Pregnancy outcomes of dams treated with cadmium from GD 12 to GD 19"

Parameter	Control	0.5 µg Cd	5.0 µg Cd
Number of dams	7	8	8
Pregnancy length (days)	22.17 ± 0.26	22.21 ± 0.21 (0.18)	22.32 ± 0.27 (0.67)
Litter size	11.51 ± 1.12	12.08 ± 1.37 (4.95)	11.67 ± 1.35 (1.39)
Pup birth weight (g)	5.92 ± 0.24	5.68 ± 0.31 (-4.05)	5.91 ± 0.23 (-0.16)
Viability index (%)	100.00 ± 0.00	96.48 ± 4.72 (-3.52)	100.00 ± 0.00 (0.00)
Weaning index (%)	92.58 ± 5.10	90.00 ± 9.05 (-2.78)	99.04 ± 0.96 (6.99)

Values are expressed as mean ± S.D. Values in parentheses are % change from control.

Table No. 2: "Duration of different stages of estrous cycle (days) in control rats and rats exposed to 0.5 and 5.0 µg Cd/kg body weight during embryonic development"

Estrous stage	Control	0.5 µg Cd	5.0 µg Cd
Pro-estrous	1.61 ± 0.05	1.03 ± 0.05 (-36.02)	1.01 ± 0.01 (-37.26)
Estrous	0.82 ± 0.30	0.92 ± 0.14 (12.19)	0.79 ± 0.04 (-3.65)
Met-estrous	1.71 ± 0.20	1.39 ± 0.30 (-18.71)	1.03 ± 0.01 (-39.76)
Di-estrous	1.92 ± 0.32	1.80 ± 0.26 (-6.25)	1.39 ± 0.24 (-27.60)
Cycle duration	6.06 ± 0.21	5.14 ± 0.18	4.22 ± 0.07

Values are average of three sequential estrous cycles from 18 individual females.

Table No. 3: "Reproductive performance of control rats and female rats exposed to 0.5 and 5.0 µg Cd/kg body weight during embryonic development"

Parameters	Control	0.5 µg Cd	5.0 µg Cd
Conception time (days)	1.43 ± 0.19	1.37 ± 0.23 (-4.19)	1.45 ± 0.22 (1.39)
Mating index (%)	100 (18/18)	100 (18/18)	100 (18/18)
Fertility index (%)	100 (18/18)	100 (18/18)	100 (18/18)
Number of corpora-lutea/rat	14.21 ± 2.12	14.78 ± 2.07 (4.01)	16.01 ± 1.02 (12.66)
Number of implantations /rat	14.07± 2.11	14.17± 2.01 (0.71)	15.78± 2.11 (12.15)
Pre-implantation loss (%)	0.98	4.13	1.44
Number of live fetuses/rat	12.31 ± 1.37	13.78 ± 1.71 (11.94)	13.79 ± 1.82 (12.02)
Post-implantation loss (%)	12.50	2.75	12.61

Values are mean ± S.D. of 18 individuals Values in parentheses are percent change from that of control.

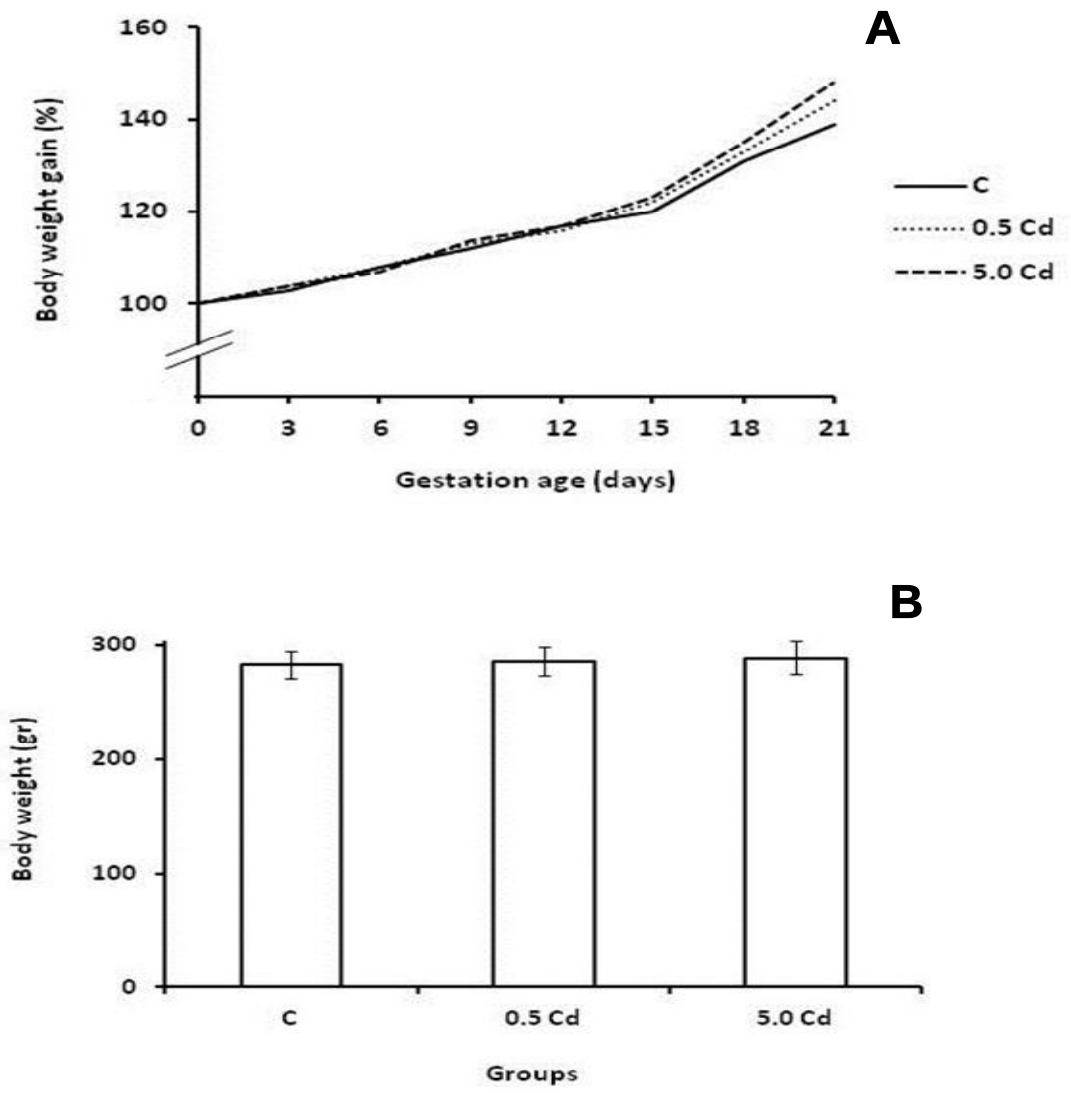

Figure No. 1: Body weight gain in dams exposed to cadmium during pregnancy (A) and corrected body weight on GD 21 (B).

The dams were treated with 0 (control), 0.5 or 5.0 mg Cd /Kg B.W. from GD 12 to GD 19. Body weight gain was determined by considering the body weight of the dams on GD 0 as 100%. The body weight of the dams on GD 21 was corrected by subtracting the sum of the weights of the pups on post-natal day 1. The data are expressed as mean ± S.D. n = 7, 8 and 8 for control, 0.5 mg Cd and 5.0 mg Cd groups respectively.

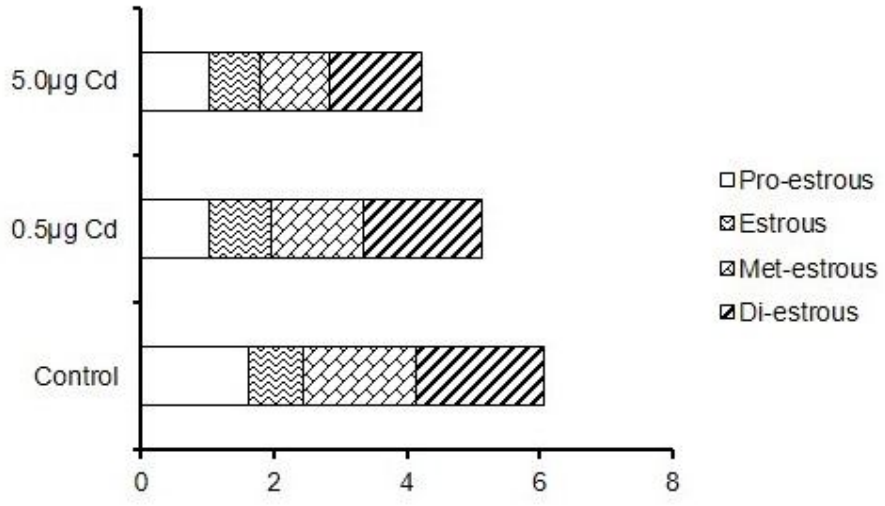

Figure No. 2: Duration of estrous cycle (days) in control rats and rats exposed to 0.5 and 5.0 µg Cd/kg body weight during embryonic development

Values are average of three sequential estrous cycles from 18 individual females

4. CONCLUSIONS

Taken together, the results of the present study unveiled the effects of gestational exposure to Cd on fertility output and reproductive efficiency of female progeny. The results indicated that exposure to Cd during pregnancy at dose level of 0.5 or 5.0 µg/kg body weight exerted no significant effect on pregnancy output as evidenced by gestation length, litter size and pup birth weight. Similarly, fertility efficiency of female progeny of cadmium exposed dams also not affected since the numbers of corpora lutea, conception time, mating and fertility indices, number of live fetuses were comparable with controls. Conversely, male offspring of cadmium exposed dams exhibited reduced spermatogenesis and steroid genesis and fertility efficiency (authors' unpublished data). Therefore, considering the current levels of cadmium pollution in several places in nature and the increased incidence of infertility in the population might be due to harmful effects of cadmium on male reproduction.

5. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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