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Effects of Chronic Exposure to Cobalt Chloride on the Fertility and Testes in Mice

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Abstract

The effect of twelve weeks ingestion of cobalt chloride ($CoCl_2$) was investigated on the fertility of adult male Swiss mice. Sexually mature male mice were exposed to 200, 400 and 800 ppm cobalt chloride dissolved in drinking water. Based on fluid consumption, exposed animals received an average of 25.66 ± 2.34, 46.91 ± 4.78 and 93.01 ± 6.76 mg/kg/day cobalt chloride, respectively. After the exposure period, these males were mated with untreated female mice. Fertility was significantly reduced in males that ingested cobalt chloride. The number of pregnant females and the number of implantation sites were significantly reduced in females mated with males that had ingested $CoCl_2$ at a concentration of 400 or 800 ppm. The total number of resorptions and the number of females with resorptions were significantly increased in females mated with males that had ingested $CoCl_2$ at all concentrations. On the other hand, the number of viable fetuses was decreased in females impregnated by exposed males at the three concentrations. Absolute epididimal weight, and the relative or absolute testes weights were significantly reduced in males that ingested cobalt chloride at a concentrations of 400 or 800 ppm. In contrast, absolute and relative weights of the seminal vesicles were significantly increased. Furthermor, epididymal and sperm counts and daily sperm production were significantly decreased in males that ingested $CoCl_2$. Histological evaluation of the testes revealed several abnormalities including hypertrophy of the interstitial Leydig cells, congested blood vessels, degeneration of the spermatogonial cells and necrosis of both the seminiferous tubules and the interstitial tissue.

Key words: $CoCl_2$, Swiss mice, reproductive system

INTRODUCTION

The reproductive and developmental effects of heavy metals are of considerable interest due in part to their effects on human fertility [1,2]. Cobalt, a natural element present in certain ores of the earth's crust, is essential to life in trace amounts, but excess dietary cobalt causes toxic effects in mammals [3]. Everyone is exposed to low levels of cobalt in air, water and food. Previous work has indicated that exposure to cobalt might cause adverse effects on the male reproductive system [4]. In studying the effects of cobalt on male rat reproduction Hoey [5] observed testicular necrosis of both the seminiferous tubules and testicular interstitial tissue after subchronic exposure to cobalt via daily injections of 0.40 mmole/kg body weight over a 30 day period. Testicular atrophy was demonstrated after chronic oral exposure of male rats to cobalt (20 mg/kg body weight) for 69 day [6]. Chronic cobalt treatment (20 mg/kg body weight) of rats via ingestion caused depletion of live sperm and produced toxic effects on the germinal epithelium [7]. In another study, it was shown that chronic exposure to cobalt dramatically affected male mice fertility in a time-and dose-dependent manner, while acute administration had minimal effects [4]. Likewise, continuous exposure of male mice to cobalt (400 ppm) via drinking water over a 13-week period resulted in a reproducible, sequential pattern of seminiferous tubule degeneration [8]. Inhalation of the soluble cobalt sulfate (3 mg/m^3) for 13 weeks caused reduce sperm motility in mice, and at a higher concentration (30 mg/ m^3), the number of abnormal sperm was increased, while the testis and epididymal weights were decreased [9]. On the other hand, oral administration of cobalt chloride to pregnant rats at 12, 24, and 48 mg/kg body weight/day from day 14 of gestation through day 21 of lactation significantly affected the postnatal survival and development of the pups [10]. In contrast, exposure of pregnant rats to cobalt chloride by daily gavage at doses of 25, 50, or 100 mg/kg body weight on gestation days 6-15 had no significant effects on the number of corpora lutea per dam, implantations per dam, resorptions and dead fetuses per litter, or the number of live fetuses per litter [11].

Occupational exposure to various cobalt compounds is of concern because of thier mutagenic [12, 13] and carcinogenic [14, 15] effects. The toxic effects of cobalt have been observed in several organ systems. Polycythemia, goiter, cardiomyopathy, hyperglycemia, allergic dermatitis, and respiratory impairment have been linked to chronic exposure to cobalt [16]. Since cobalt and its salts are widely used in industry as components in paints, grinding wheels, hygrometers and electroplating, varnishes, in vitamin B12, as a foam stabilizer in beer and as a catalyst in the petrochemical industry [17] it is likely that many workers are exposed to it. The aim of the present work is to further investigate the effects of cobalt administered in drinking water on fertility of male mice.

MATERIALS AND METHODS

Animals

Forty adult male Swiss mice, at day 60 of age, weighing approximately 32 g were used in this study. They were raised in the animal house unit in the Faculty of Medicine at Jordan University of Science and Technology under a controlled temperature of 21±1°C on a 12 h light, 12 h darkness schedule (lights on 06.00-18.00h). Food (manufactured by the Faculty of Veterinary Medicine at Jordan University of Science and Technology, Irbid, Jordan, according to standard recipes) and water were offered *ad libitum*. Jordan University of Science and Technology ethical comitee has approved performing this experiment on mice.

Administration of cobalt chloride

Cobalt chloride hexahydrate ($CoCl_2.6H_2O$, M.W. 237.95) [Sigma Chemical Company, St Louis, MO, USA] was dissolved in tap water at a concentration of 200, 400, and 800 ppm. Male mice were randomly assigned into groups of ten animals each and allowed *ad libitum* access to tap water containing cobalt chloride for a period of 12 weeks. Control male mice were given tap water without any added cobalt chloride. Based on fluid consumption, the mice exposed to 200, 400 or 800 ppm cobalt chloride solutions received an average dose of 25.66 ± 2.34, 46.91 ± 4.78 or 93.01 ± 6.76 mg/kg body weight, respectively. It is of interest to point out that our calculations of cobalt intake are based solely on the cobalt chloride added to the drinking water.

Fertility test

Animals were observed daily from the first day of exposure to cobalt chloride for clinical signs of toxicity. Water consumption was measured every day and body weights every week. After the exposure period, each male was caged with two virgin untreated females of the same strain and given ad libitum access to food and untreated tap water. They were left together for 10 days during which two estrus cycles should have elapsed [18]. Adult male mice that ingested cobalt chloride as well as the control males, were then removed and sacrificed for further evaluations. Ten days later, the mated females were killed by cervical dislocation under light ether anesthesia and the following measurements were recorded: number of pregnant females, number of implantation sites/female, number of viable fetuses/female, number of resorptions, and number of females with resorptions.

Procedures employed in the use and sacrifice of mice were in accordance to the NIH Guide for the Care and Use of Laboratory Animals.

Evaluation of reproductive organs weights

Cobalt-exposed and control males were sacrificed after twelve weeks of cobalt chloride ingestion and the 10 day period of mating. The following organs were excised and weighed: paired testes, seminal vesicles (stripped of seminal fluid), epididymides and preputial glands.

Testicular and epididymal sperm counts

The excised left testis and epididymis were weighed. The testis from each mouse was placed in 10 ml of 0.9% sodium chloride (normal saline) and refrigerated for later homogenization for spermatid count. The epididymis was placed in 10 ml of normal saline and refrigerated for later homogenization for epididymal sperm count.

Sperm count was performed according to the method of Amann and Lambiase [19]. Briefly, the excised left testis or epididymis from each mouse was sectioned by a disposable blade in 4 ml of normal saline in a conical glass petri dish, and then minced using a manual glass homogenizer. The homogenate was mixed using a vortex mixer and the number of sperm measured using a hemocytometer. Epididymal sperm counts were expressed as number of sperm/mg epididymis. Testicular spermatid counts were expressed as the number of spermatids/g testis. The estimate of daily sperm production/testis/day was calculated based on a factor of 4.84, which is the duration of a seminiferous cycle during which developing spermatozoa are in the spermatid stage as described by Vom Saal *et al.* [20]

Histological evaluation of testes

The excised testis was fixed in a10% formalin solution and then processed using standard histological procedures. The tissue was embedded in paraffin blocks, sectioned perpendicular to the longest axis of the testis at 7 μm thickness and stained with hematoxylin and eosin. Stained sections were mounted with distyrene plasticizer xylene (DPX) and examined using light microscopy.

Statistical analysis

Data are expressed as mean ± S.D. Differences between control and test groups were analyzed using either Student's't' test or Fisher's exact test using StatMost 2.5 Windows software/DataMost Corporation. A *p*-value less than 0.05 were considered significant.

RESULTS

Effect of cobalt chloride on body weight gain and fluid consumption

The data presented in Table 1 demonstrate that ingestion of cobalt chloride (200, 400 or 800 ppm concentration) reduced the the average body weight gain in the test animals ($p<0.01$). The average daily fluid consumption per animal was also reduced ($p<0.0001$). The average doses of cobalt chloride that the animals received based on fluid consumption/kg body weight/ day are presented in Table 1. In the exposed groups, two animals out of 10 and one out of 10 died during the 10^{th} weeks of the exposure to 800 and 400 ppm cobalt chloride, respectively. There were no other signs of clinical toxicity observed in the survived animals.

Effect of cobalt chloride on fertility

The results presented in Table 2 demonstrate the adverse effects of cobalt chloride on male mouse fertility. The number of females that became pregnant was reduced when they mated with males exposed to 400 ppm ($p<0.05$) or 800 ppm ($p<0.001$) cobalt chloride. The number of implantations was reduced in females impregnated by males that had ingested 200 or 400 ppm cobalt chloride ($p<0.01$). Furthermore, the number of viable fetuses was also reduced in pregnant females impregnated by males exposed to 200 ($p<0.01$), 400 ($p<0.001$) and 800 ppm

Table 1. Effect of 12 weeks ingestion of cobalt chloride on body weight gain and water consumption of adult male mice.

Treatment (ppm)	Body weight (g)[a]	Fluid intake (ml)[a]	Average dose (mg/kg body weight/d)[a]
Control (tap water)	35.27 ± 2.14	6.23 ± 0.40	0
Cobalt chloride (200)	33.59 ± 2.75*	4.31 ± 0.39***	25.66 ± 2.34
Cobalt chloride (400)	33.00 ± 1.58*	3.87 ± 0.39***	46.91 ± 4.78
Cobalt chloride (800)	32.77 ± 1.30**	3.81 ± 0.28***	93.01 ± 6.76

[a] Results are expressed as mean ± S.D.
* p<0.01 **p<0.005, ***p<0.0001 (Student *t* test).

Table 2. Effect of 12 weeks ingestion of cobalt chloride via drinking water on fertility of adult male mice.

Treatment (ppm)	Number of males	Number of mated females	Number (%) of pregnant females	Number of implantation sites[a]/female	Number of viable fetuses[a]	Total Number of resorptions/ Total No. of implantation sites	Number (%) of animals with resorptions
Control (Tap water)	10	20	19/20 (95.0)	7.89 ± 2.38	7.74 ± 2.40	3/150	3/19 (16)
Cobalt chloride (200)	10	20	15/20 (75.0)	5.67 ± 2.02^{++}	5.00 ± 2.14^{++}	9/81***	10/15** (67)
Cobalt chloride (400)	9	18	12/18 * (66.7)	5.42 ± 1.68^{++}	4.67 ± 1.83^{+++}	9/65***	10/16** (63)
Cobalt chloride (800)	8	16	7/16*** (43.8)	6.43 ± 2.23	5.83 ± 1.94^{+}	10/45****	5/7* (70)

[a] Results are expressed as mean ± S.D.
$^{+}$ p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 (Student *t* test).
* p<0.05, **p<0.005, ***p<0.001, ****p<0.0001 (Fisher's exact test).

Table 3. Effect of 12 weeks ingestion of cobalt chloride via drinking water on reproductive organ weights of adult male mice[a].

Treatment (ppm)	Number of Males	Epididymis weight (mg)	Testes weight (g)	Seminal vesicles weight (g)	Preputial gland weight (g)
Control (Tap water)	10	32.31± 1.66	0.21 ± 0.01 (57.75 ± 3.89)	0.13 ± 0.02 (36.36 ± 6.82)	0.096 ± 0.01 (26.07 ± 2.51)
Cobalt chloride (200)	10	31.88 ± 1.46	0.19 ± 0.01** (55.45 ± 3.18)	0.13 ± 0.03 (35.57 ± 7.46)	0.107 ± 0.015 (30.44 ± 4.86)*
Cobalt chloride (400)	9	31.75 ± 1.45	0.18 ± 0.01*** (53.08 ± 3.33)*	0.20 ± 0.02** (61.91 ± 8.24)***	0.096 ± 0.016 (29.32 ± 5.90)
Cobalt chloride (800)	8	29.80 ± 0.93***	0.15 ± 0.02 **** (46.28 ± 7.31)***	0.23 ± 0.07 *** (68.32 ± 21.94)***	0.094 ± 0.023 (28.81 ± 7.13)

[a] Results are expressed as mean ± S.D.
Relative organ weights, expressed as mg/10g body weight.
* p<0.05, **p<0.01, ***p<0.005, ****p<0.0001 (Student *t* test).

Table 4. Effect of 12 weeks ingestion of cobalt chloride via drinking water on testicular and epididymal sperm counts in adult male mice.

Treatment (ppm)	Epididymal Sperm Count/mg Epididymis[a] (X 10^3)	Testicular Sperm Count/g testis[a] (X 10^3)	Daily Sperm Production/Testis[a] (X 10^5)
Control (Tap water)	192.79 ± 15.68	44.94 ± 2.72	10.12 ± 0.74
cobalt chloride (200)	167.23 ± 23.10*	45.36 ± 3.11	9.77 ± 1.20
cobalt chloride (400)	166.23 ± 25.41*	34.78 ± 2.31**	6.50 ± 0.39 ***
cobalt chloride (800)	150.62 ± 12.40***	33.31 ± 2.23 ***	5.81 ± 0.42 ****

[a] Results are expressed as mean ± S.D.

* p<0.05, **p<0.01, ***p<0.005, ****p<0.0001 (Student *t* test).

(p<0.05) cobalt chloride. In addition, the total number of resorptions and the number of animals with resorptions were increased in females mated with the exposed males at the three concentrations of cobalt chloride.

Effects of cobalt chloride on weights of reproductive organs

The data presented in Table 3 show the effect of exposure to cobalt chloride on weights of male reproductive organs. Absolute epididymal weights were decreased in males exposed to cobalt chloride at a concentration of 800 ppm cobalt chloride (p<0.005). Testes weights were significantly decreased in males that were exposed to all three concentrations of cobalt chloride. Furthermore, mice exposed to 400 and 800 ppm for 12 weeks had a statistically significant increase in the absolute weight of seminal vesicles (p<0.001 and p<0.005, respectively). The relative weights of preputial glands were increased in males exposed to 400 ppm cobalt chloride (p<0.05).

Effect of cobalt chloride on testicular and epididymal sperm counts

Table 4 presents testicular and epididymal sperm counts and daily sperm production of male mice in the control and the test groups. Epididymal sperm counts were decreased in male mice exposed to 200 ppm, 400 ppm (p<0.05) or 800 ppm (p<0.005) cobalt chloride. Similarly, testicular sperm counts were decreased in the groups that exposed to 400 (p<0.01) or 800 ppm (p<0.005) cobalt chloride. Daily sperm production was decreased in males that ingested cobalt chloride at a concentration of 400 (p< 0.005) or 800 ppm (p<0.0001).

Effect of cobalt chloride on the histology of testis.

Histological sections of the testis were examined to determine whether the reduction in the fertility of treated mice was in part due to a direct effect of cobatous chloride on the structure of the testes. In general, all sections of testes collected from mice administered 400 and 800 ppm cobalt chloride had necrosis of both the seminiferous tubules and the interstitial tissue, congested blood vessels, hypertrophy of the interstitial Leydig cells and degeneration of the spermatogonial cells (Figs. 2 and 3). Control mice didn't have these histological abnormalities (Fig.1).

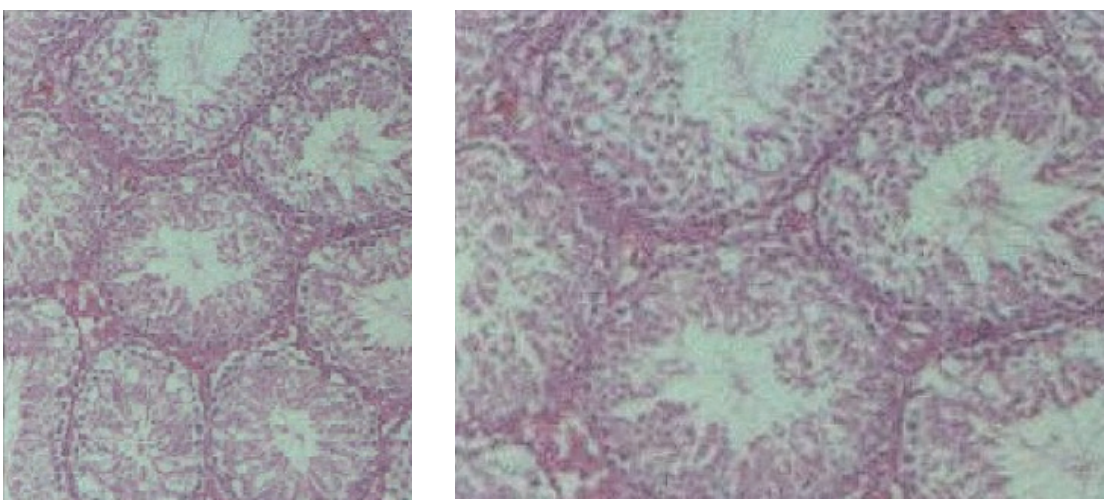

Figure 1. Cross section of the seminiferous tubules in the testis of a control mouce. A: Notice regularly arranged tubules with no necrosis. Magnification: 80X. B: Higher magnification of A (205X).

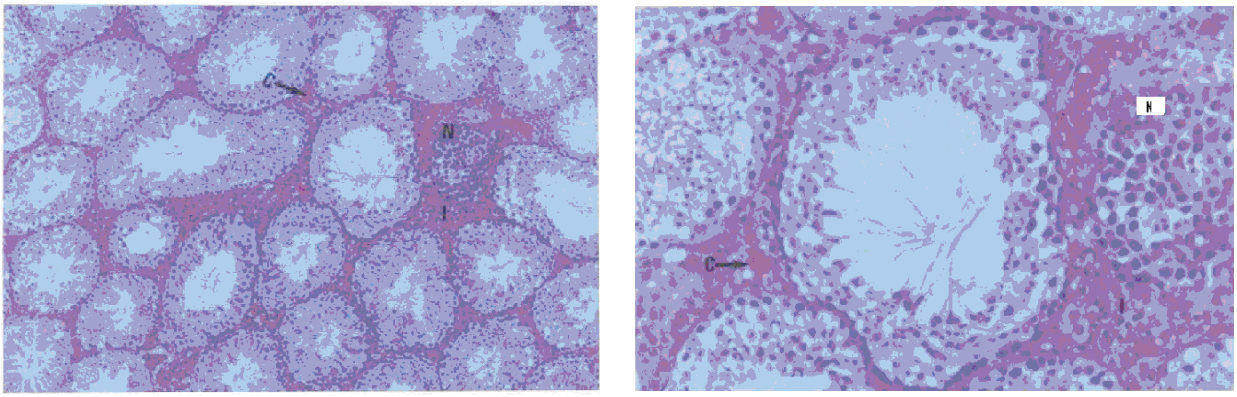

Figure 2. Cross section of the seminiferous tubules in the testis of a mouse administered 800 ppm cobalt chloride. A: Notice necrosis (N) and interstitial tissue (I) and congested blood vessels (C). B: Higher magnification of A (205X).

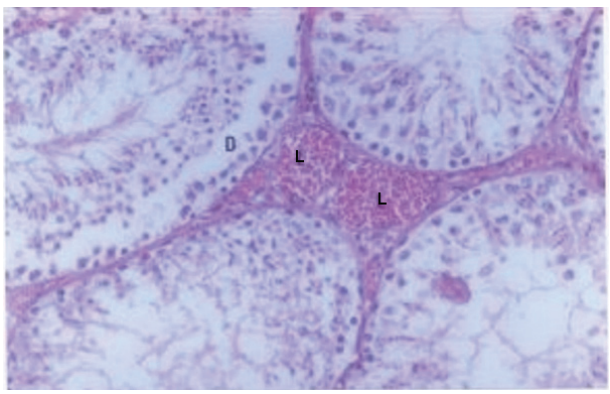

Figure 3. Cross section of testis of a mouse administered 800 ppm cobalt chloride. Notice enlargement of the interstitial Leydig cells (L) and degeneration (D) of spermatogonial cells of the seminiferous tubules Magnification: 205X

DISCUSSION

The aim of the present study was to assess the adverse effects on fertility of male mice after ingestion of cobalt chloride via drinking water. The concentrations of cobalt chloride employed in this study were chosen after consideration of previous studies [4, 10].The results presented in this study demonstrate that ingestion of cobalt chloride for 12 weeks caused a significant decrease in the average body weight gain of test males (Table 1). This reduction in body weight gain is an indication of general toxicity. However, it has been shown that the reproductive system of male mice was relatively resistant to body weight decreases down to even 70% of the body weight of control animals [21].

Our work showed that long-term exposure of adult male mice to cobalt chloride adversely affected various fertility parameters. This impairment in male fertility was dose-dependent and increased as the concentration of cobalt chloride increased. Measurement of testicular and epididymal weights, weights of secondary sex glands, sperm counts and fertility as well as the histology of the testes in our study suggest a direct effect of cobalt on the seminiferous tubules and the surrounding interstitial tissue. The observed necrosis in the seminiferous tubules and the interstitial tissue in our study is in agreement with previous morphologic studies of the rodent testis [5, 7]. The hypertrophy of the interstitial cells of Leydig was in contrast to previous report by Hoey [5] who observed necrotic changes in the interstitial cells and the seminiferous tubules. The increase in the size of the interstitial Leydig cells and possibly the activity of these cells could explain the significant increase in the weight of the seminal vesicles observed in cobalt treated mice. It is known that the size of secondary sex organs is dependent on the concentration of testosterone and dihydrotestosterone. In an attempt to determine the cause of fertility impairment in male mice after chronic exposure to cobalt, Pedigo *et al.* [4] reported an elevated concentration of serum testosterone, while the concentrations of gonadotropins (leutinizing hormone [LH] and follicular stimulating hormone [FSH] remained unchanged. It has been suggested that cobalt indirectly affects testosterone production by interfering with local inhibitory feedback mechanisms [4]. An inhibitory factor produced within the seminiferous tubules has been demonstrated to decrease testosterone production by Leydig cells [22].

The data presented in this work demonstrate that chronic exposure of adult male mice to cobalt caused a significant decrease in the weights of testis and epididymis. Decreased testicular and epididymal weights are an indication of reduced spermatogenesis. This reduction in spermatogenesis is likely the cause of decrease fertility. Our results indicate a significant decrease in male fertility as measured by the number of females that became pregnant after mating with the treated males.

The results showed that ingestion of cobalt caused a significant decrease in the number of implantations and a significant increase in the number of resorptions in females impregnated by the test males. These effects may be attributed to poor development of fertilized ova due to alterations in sperm quality. A significant depression in sperm motility was reported after chronic exposure of male mice to cobalt [4]. Furthermore, our work has shown that the weight of the preputial gland was significantly increased in test males that ingested cobalt chloride at a concentration of 400 ppm. This increase in the weight of preputial glands seemed to be inhibited at 800 ppm. It has been noted that the size and activity of the preputial gland in rodents are clearly influenced by a variety of steroid hormones [23]. Preputial glands also produce behavior-modulating phermones that alter fighting and other behaviors in rodents [24].

In summary, our work demonstrated that cobalt chloride administration in drinking water at the doses used in this study for a period of 12 weeks caused adverse effects on the fertility of male mice.

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