EFFECT OF COBALT ON MALE REPRODUCTIVE ORGANS DURING PUBERTY

M. Madzharova, Y. Gluhcheva, E. Pavlova and N. Atanassova
Bulgarian Academy of Sciences, Institute of Experimental Morphology and Anthropology with Museum, Sofia, Bulgaria
Correspondence to: Ekaterina Pavlova
E-mail: e_bankova@yahoo.com

ABSTRACT
Cobalt is an essential oligoelement for mammals. It is not a cumulative toxin but chronic exposure induces negative effects on the organism. Data from the literature evidenced that in experimental animals cobalt impaired male reproductive organs and fertility when applied chronically. The aim of our study is to follow the effect cobalt on pubertal male progeny of female mice treated with cobalt in late pregnancy and during suckling period. Macroscopic parameters as weight of male reproductive organs and organ/body weight ratio were established. Significant reduction in body weight and 20% decrease (non significant) of testicular and epididymal weight as well as in testis/body weight index was found. The negative effect of cobalt on male progeny could be explained with transplacental route of exposure and with possible transfer of cobalt into mothers’ milk. The negative effect of cobalt was not seen in mid puberty (day 25) with the exception of epididymal weight which was not compensated suggesting that epididymis is more sensitive to cobalt treatment. In conclusion, our data indicate that exposure to cobalt during perinatal and postnatal period affected body weight during puberty but not significantly reduced reproductive organs growth. However, negative impact of cobalt on later life could not be rule out and cobalt might be considered as possible risk factor for male reproductive health.

Keywords: male, puberty, reproductive organs, cobalt

Introduction
Cobalt is an essential oligoelement for mammals. It resides in the body as a constituent of cobalamin (vitamin B\textsubscript{12}), mainly. The adult human body contains approximately 1mg of cobalt, 85% of which is in the form of Vitamin B\textsubscript{12}. Human dietary intake of cobalt varies between 5 and 50g/day (6). Food and beverages represents the main source of cobalt for the general population (7). In occupational settings, workers are exposed to higher cobalt concentrations in the form of dust, and for this target group the intake of cobalt by inhalation and skin absorption represents a significant source of cobalt (4). Cobalt is not a cumulative toxin and excretes rapidly by urine (8) and to a lesser extent via faeces (7). The presence of cobalt in blood and urine usually reflects recent cobalt exposure (8). It was proven that cobalt passes via placenta appearing in the fetal blood and amniotic fluid and it is shown to possess an embryotoxic effect (16). Cobalt is reported to exert genotoxic and carcinogenic effect as well. The negative effect of cobalt is attributed to its ability to generate oxidative stress that in turn impaired DNA repairing mechanisms and induce cell apoptosis (4). Cobalt possesses an allergic potential it is considered as the second of top five global allergens (10). Chronic exposure to cobalt leads to its accumulation in different tissues and organs that could induce negative effects for the entire organism. Respiratory organs, the skin, the hematopoietic tissues, the myocardium, the thyroid gland are targets of cobalt action (13). High level of cobalt was detected in blood, liver, kidney, testes, epididymis, bone, brain, liver in experimental animals treated with cobalt (3, 12, 14).

Experimental treatment with cobalt exerts negative effect on male reproductive organs and fertility when applied chronically (1, 2, 5, 9, 11, 14) whereas acute administration has minor effect (15). It was established that cobalt impaired male fertility: preimplantation losses, fetus resorptions, less
pregnancies, decreased number of viable fetuses and live births were established after cobalt treated males mated with untreated females (5, 14). Data evidenced for reduction of testicular and epidydimal weight, epididymal sperm concentration, depressed sperm motility and increased number of abnormal sperm (1, 2, 5, 15). Moreover, cobalt treatment during adulthood caused degeneration of seminiferous epithelium, manifested by vacuolation of Sertoli cells, formation of abnormal spermatid nuclei and shrinkage of the tubules. Folding and thickening of basal laminae, hypertrophy of Leydig cells, enlargement of interstitial area and dilatation of blood capillaries were detected (1, 5, 9, 11). Morphometric analysis revealed decreased volume of seminiferous epithelium whereas the volume of interstitium, the diameter of lumen and seminiferous tubules were significantly increased (9). Hormonal balance in cobalt treated males was also impaired manifested by dramatically increased serum testosterone levels while LH and FSH serum levels remained normal (15).

Data in the literature are mostly generated from experimental models with cobalt treatment during adulthood. The influence of cobalt in early stages of development is poorly investigated. Crucial events occurred during establishment of first spermatogenesis. In this respect our study aimed to observe the effect cobalt on pubertal male progeny of female mice treated with cobalt in late pregnancy and during the suckling period. We focussed our study on the effect of cobalt chloride on pubertal male pups and they were divided into two groups – early and mid puberty, sacrificed at 18 postnatal day (pnd) and 25 pnd respectively. Each experimental group was consisted of 6 to 13 animals. Testes and epididymides were sampled, weighted, immersed in Bouen’s fixative and embedded in paraffin using routine histological practice. The data obtained were statistically processed using Student’s t-test and difference was considered significant at p<0.05.

Results and Discussion

Fig. 1a and Fig. 1b represent the body weight of male pups in early and mid puberty - 18 pnd and 25 pnd respectively at the two doses applied – 75 mg/kg (low dose) and 125 mg/kg (high dose). Significant decrease in the body weight on 18 pnd in both treatment groups was found. On 25 pnd this negative effect of cobalt was not seen and differences between treatment and control groups were non significant. The negative effect of cobalt in early puberty (day 18) could be explained by the transplacental route of action (16) during pregnancy. Another possible way of exposure is via mothers’ milk, as it was established that in human metal ions transferred into mothers’ milk (17). The restoration of body weight in mid puberty (day 25) could probably reflects some recovery mechanism as dose applied on mothers became less per body weight of the growing pups on day 25 compared to day 18.

Fig. 1. Body weight on 18 (A) and 25 postnatal day (B) after perinatal and postnatal treatment with 75 mg/kg and 125 mg/kg cobalt chloride
**Fig. 2a** and **Fig. 2b** represents testicular weight of male pups on day 18 and 25. In our experiment we did not find any significant difference between both treatment groups and the controls although the mean values were lower by 20%. The decrease of testicular weight could be explained with the transplacental route (16) and possibly mothers’ milk (17) of exposure to cobalt chloride. In the mid puberty (day 25) testis weight in both treatment groups recovered almost to the control value.

![Testis weight bars](image)

**Fig. 2.** Testis weight on 18 (A) and 25 postnatal day (B) after perinatal and postnatal treatment with 75 mg/kg and 125 mg/kg cobalt chloride

Epididymal weight on 18 pnd is presented on **Fig. 3a** and decrease by 20-30% in treated animals was found when compared to controls although the difference was no significant. On 25 pnd (**Fig. 3b**) the epididymal weight remained no significantly decreased by 20% in comparison with control group. As we mentioned above, the negative effect of cobalt on body and testis weight, found on 18 pnd was not seen on 25 pnd suggesting that epididymis is more sensitive to cobalt treatment compared to the testis.

![Epididymis weight bars](image)

**Fig. 3.** Epididymis weight on 18 (A) and 25 postnatal day (B) after perinatal and postnatal treatment with 75 mg/kg and 125 mg/kg cobalt chloride

The testis/body weight index was calculated as ratio between testicular weight to body weight. The parameter decreased by 25-30% in both treatment groups on day 18 although the differences with control value were no significant (**Fig. 4a**). On 25 pnd the testis/body weight index of cobalt treated males recovered to control value (**Fig. 4b**).

Our study provides original data concerning the effect of cobalt chloride on the progeny of treated with cobalt chloride mice during late gestation and suckling period. Comparison of our results on 18 and 25 pnd suggested that reproductive organs in early pubertal period appeared to be more sensitive to cobalt treatment in comparison with mid puberty. Future studies are planned to investigate the effect of cobalt chloride on male progeny during late puberty and adulthood as well.
**Fig. 4.** Testis/body weight index on 18 (A) and 25 postnatal day (B) after perinatal and postnatal treatment with 75 mg/kg and 125 mg/kg cobalt chloride

**Conclusions**

In conclusion, our data indicate that exposure to cobalt during perinatal and postnatal period affected body weight during puberty but not significantly reduced reproductive organs growth. However, negative impact of cobalt on later life could not be ruled out, as well as potential synergetic effect of cobalt with other heavy metals. In this regard cobalt might be considered as a potential risk factor for male reproductive health.

**Acknowledgment**

This work is supported by grant No DO02 – 351/2008 for Young scientists from the Bulgarian National Science Fund.

**REFERENCES**


