Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 16
Sofia • 2010

Influence of cobalt on male fertility

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Cobalt is an essential oligoelement for mammals in the form of cobalamin (vitamin B_{12}). Cobalt does not accumulate in the organism but high doses of cobalt could exert adverse effects.
The present article is focused on the negative influence of cobalt on male fertility. Significant reduction in epididymis and testis weight was found in cobalt treated animals. Genotoxic effects of cobalt involved significant increase in the frequency of chromosomal aberrations in male gametes. Impaired fertility was manifested by low sperm count, decreased motility, accompanied by morphological abnormalities. Structural alterations include enlargement of interstitium, desorganisation of peritubular area and degeneration of seminiferous epithelium (vacuolation of Sertoli cells, multinuclear germ cells, containing degenerative spermatocytes and spermatids). Cobalt interferes with the hormonal balance as well.

In conclusion, cobalt exposure could be considered as a risk factor for male reproductive development and function and hence for male reproductive health.

Key words: cobalt, male fertility, reproductive organs, spermatogenesis.

1. General role of cobalt

Cobalt is a naturally occurring, relatively rare element of the earth's crust [8, 14]. It circulates in surface environment through many natural processes and anthropogenic activities. Cobalt is an essential oligoelement for mammals involved as a constituent of vitamin B_{12} (cobalamin), mainly. Congenital disturbances related to absorption and function of vitamin B_{12} give rise to pathological alterations such as megaloblastic anemia, retardations, neurological and ocular defects and other syndromes and diseases [10]. Cobalt is found in very small amounts in food although fish and sea foods, meat, eggs, liver and other animal products are relatively rich in cobalt [27]. The adult human body contains approximately 1 mg of cobalt, 85% of which is in the form of vitamin B_{12}. Human dietary intake of cobalt varies between 5 and 50 mg/day [14].

There are three ways of cobalt intake — by food and drinks, by inhalation and by skin absorption. Ingestion of cobalt by food and beverages represents the main source of cobalt for human general population. Absorption of vitamin B_{12}

from food under physiological conditions involves no less than five separate vitamin B_{12}-binding molecules, receptors and transporters and each molecule has separate affinity and specificity for vitamin B_{12} and a separate cell receptor, as well [21]. Initially in the stomach vitamin B_{12} is bounded by heptacorrin. After that in ileum (the only place of vitamin B_{12} absorption) vitamin B_{12} bounds to intrinsic factor before being absorbed by the intestinal epithelial cells. Transportation into all other cells is possible only after preliminary proteolitical release of vitamin B_{12} and its subsequent binding to another transport protein — transcobalamin II [10, 21]. By blood circulation cobalt could be delivered and subsequently accumulated in different organs — most significant amount is accumulated in liver and kidneys, but higher doses of cobalt are detected in hematopoietic organs, brain, reproductive organs etc. Increased uptake of cobalt by inhalation is typical for workers in specific occupational settings such as alloys and metals manufacturing, diamond polishing, dental laboratory materials production etc [13, 14]. These workers are exposed to dust full of cobalt and other metals and hence combined effect of these elements couldn't be rule out. Main target of cobalt is respiratory system. Chronic exposure to high cobalt concentrations in the working environment leads to impaired lung function - asthma, hard metal lung disease and predisposition to lung cancer. Skin absorption rarely occurs, for example by jewelry. Cobalt has relatively high allergic potential being one of the five top global allergens [16]. Exposure to cobalt could give rise to allergic reactions and contact dermatitis [25].

Prolonged exposure to cobalt leads to different pathological alterations such as cardiomyopathy, impaired function of thyroid gland and liver. Cobalt has been shown to exert genotoxic and carcinogenic effect. Embryotoxic activity was also revealed due to transplacental route. Experimental treatment of cobalt results in increased incidence of total growth retardation, embryolethality and severe congenital abnormalities [24]. Apart its potential toxic effect, cobalt is not cumulative toxin and it is rapidly excreted in urine and to a lesser extent via faeces. Concentration of cobalt in blood and/or urine is proposed as a biomarker for cobalt exposure as elevated concentrations in body fluids mainly reflects recent contamination [12, 14]. Moreover ingestion of controlled amounts of soluble cobalt compound resulted in significantly higher concentrations of cobalt in urine and blood from females compared with that from males [4]. Cobalt toxicity could be treated with Dimercaprol, $CaNa_2$-EDTA, D-Penicillamin [27].

Some cobalt-compounds were shown to possess therapeutic potential. In the past cobalt was used for treatment of anemia, due to stimulation of erythropoietin synthesis [6]. Some cobalt-based compounds possess high antiproliferative and cytotoxic activity against human lung, ovarian, colon, uterine carcinomas and against leukemia and lymphoma cells, as well [1]. Cobalt significantly reduces plasma glucose levels and body weight in streptozotocin-diabetic rats and these data opens new perspectives for diabetic treatment strategies in the future [26].

2. Role of cobalt in male reproductive function and fertility

Cobalt-treated experimental animals show different pattern of response depending on duration of exposure (acute or chronic), applied doses and the particular species' characteristics as well. It was proven that ruminants need much higher doses of dietary cobalt for conducting of normal life than the non-ruminant animals. Cobalt toxicity in ruminants is relatively rare phenomenon in comparison with the non-

ruminants due to some physiological features of vitamin B_{12} acquirement. Duration of cobalt exposure is very important for the subsequently induced abnormalities and for the following period of recovery [1]. It is also of a great importance the period of life during which the cobalt treatment was take place.

The crucial negative effects of cobalt on testis were rendered to its ability to induce conditions characterized with more or less decreased level of oxygen. Cobalt chloride is widely used pharmacological agent for inducing hypoxia. Cobalt displaces ferrous ion from haeme, resulting in reduced oxygen-binding capacity of the molecule and hence chemically simulating hypoxia [20]. There are two main hypotheses explaining the effect of cobalt-induced hypoxia and its influence on testicular vasculature. One hypothesis is that the veins and arteries become blocked due to erythrocyte packing associated with cobalt-induced disturbance of vascular permeability. The other hypothesis is that cobalt induces polycythemia (increased erythrocyte concentration), which precipitates hypoxia due to increased blood viscosity. The testis is much more susceptible to hypoxic state than the other organs and it is under constant hypoxic environment probably due to specific organization of the testis and its vasculature.

The unique coiled testicular artery and the closely applied pampiniform plexus of veins assist in achieving the lower temperature required for spermatogenesis. The coiled testicular artery also reduces the pulse height of arterial blood flow. On the other hand, testicular blood and lymphatic vessels are restricted to capsular and interstitial tissue which commonly comprises 10 to 20% of testis volume and that result in morphological restriction of the testicular vasculature [22]. In addition human testis is more sensitive to hypoxic state in comparison with other mammals due to higher level of convolutions of seminiferous tubules which lead to increased irregularity of the blood vasculature within the testes. Moreover, human testes have lower density of intertubular and peritubular capillaries as compared to other mammals. It can be suggested that any impediment to testicular blood flow will rapidly precipitate hypoxic state. Therefore these structural specificities of testicular vasculature make testis much more sensitive to induced hypoxic states. Ischemia of testis due to torsion has been shown to result in a permanent loss of spermatogenesis [20]. Smaller degree of testicular torsion did not reduce testicular secretion of testosterone whereas prolonged torsion diminished testicular steroidogenesis in man although the role of hypoxia in modulating of testicular steroidogenesis is not well studied [20].

Experimental treatment with cobalt induced a lot of abnormalities in reproductive organs that seriously affect male fertility. Experimental animals showed decreased weight of testes and epididymis while weight of seminal vesicles and preputial glands was significantly increased. Structural changes in the testis involved necrosis and degeneration of seminiferous epithelium and interstitium [3, 7]. Corrier et al. [5] found that damaged tubules often presented side by side with normal tubules. Degeneration of seminiferous epithelium was initially manifested by vacuolation of Sertoli cells, formation of abnormal spermatid nuclei and multinucleated cells that often contained degenerative spermatocytes and/or spermatids. Spermatogonia, primary spermatocytes and round spermatids were markedly affected, while elongated spermatids, and spermatozoa were more resistant to cobalt treatment and Sertoli cells were the last surviving cells [3, 5]. Cobalt chloride-induced oxidative stress leads to alteration in behavior of tesmin — a testis specific protein with stage-specific distribution and to induction of apoptotic signals in spermatocytes, as well [23]. Sloughing of germ and Sertoli cells was also found as well as formation of empty spaces within the seminiferous epithelium [15]. Bitner et al. [3] reported shrinkage of

the tubules with accumulation of "calcified" necrotic debris accompanied by disorganization of peritubular cells and folding of basal lamina.

Cobalt increased the number of abnormal spermatozoa that consequently reduced fertility in human and animals [9]. Depletion of live sperm and reduced motility of the spermatozoa was observed [7, 19]. Testicular/epididymal sperm counts and daily sperm productions were significantly decreased. The negative effect of cobalt on sperm involved head and tail abnormalities [9, 17]. The head shape abnormalities reflect changes in the DNA content while tail alterations include loss of filaments and degeneration of mitochondria.

Morphometric analysis revealed significant decrease in relative volume of seminiferous epithelium in cobalt treated animals, whereas the relative volume of interstitium was significantly increased. Diameter of seminiferous tubules was increased probably due to higher value of luminal diameter. The number of cell nuclei per defined area was also elevated [15] although heigh of seminiferous epithelium remained relatively constant. Enlargement of interstitial space was accompanied by hypertrophy of Leydig cells and thickening of testicular vessels [7]. Blood capillaries were dilated and transmission of blood elements into the interstitium was detected, indicating oedematization [15].

Experimental treatment with cobalt influenced Leydig cells steroidogenesis - serum testosterone levels were dramatically increased, while FSH and LH serum levels remained normal. Data suggests that cobalt interfere with local regulatory mechanisms in testosterone synthesis [19]. It is well known that regulation of steroidogenesis by luteinizing hormone is mediated by cAMP and calcium [11]. Cobalt (Co^{2+}) is a calcium channel blocker, and hence it could interfere with the signal transduction pathways involved in steroidogenesis. The increase in size of interstitial Leydig cells and possibly their activity could be responsible for elevated testosterone levels that in turn could explain higher weight of seminal vessels in cobalt-treated mice [19].

Impairment of male fertility as a result of cobalt treatment was demonstrated by experimental model in which untreated females were mated with cobalt-treated males, subjected to chronic cobalt treatment before mating. Impaired male fertility results in lower number of pregnant females and number of implantation sites. Moreover, the total number of resorptions and the number of females with resorptions were significantly increased [7]. The number of viable fetuses as well as the number of total and live births was decreased. The authors suggested that these effects may be attributed to poor development of fertilized ova due to alterations in sperm quality resulted from cobalt-treatment [7].

It was recognized that cobalt possesses mutagenic and carcinogenic activity. The genotoxic effect of cobalt concerning male reproduction was poorly investigated. Hassan et al. [9] established that $CoCl_2$ exerted genotoxic effect on mice somatic and germ cells, e.g. significant increase in the frequency of chromosomal aberrations in mouse spermatocytes. The mutagenic potential of cobalt and its compounds was evaluated by International Agency for Research on Cancer. Cobalt (II) compounds were reported to induce DNA damage, DNA protein cross links, gene mutation, sister chromatid exchanges, and aneuploidy in in vitro studies with animal and human cells [9].

Regarding to the direct genotoxic mechanisms, cobalt (II) induces formation of reactive oxygen species (ROS) when combined with hydrogen peroxide in cell free system and the ROS were suggested to give different kinds of site-specific DNA damage. Cobalt ions were shown to substitute for zinc in protein-zinc finger domains which control gene expression. Such substitution is suggested to generate free

radicals close to DNA which in turn caused DNA damage [9]. Some of the genotoxic effects of cobalt (II) are attributed to its activity as poison of topoisomerase II demonstrated in cultured cells [2]. It was also shown that cobalt interferes in the DNA repair processes causing their inhibition [6]. Cobalt competes with the essential Mg (II) ions [6] suggesting possible interference with the processes/biochemical reactions required Mg (II) ions.

It is important to note that acute cobalt exposure did not give rise to any significant or irreversible alterations concerning male sexual function and reproductive system whereas the consequences of the chronic exposure are much more powerful. It was reported that following acute occupational cobalt inhalation the urinary elimination is rapid for 24 h followed by a slower excretion phase lasting several weeks. The repeated dose treatment with food may result in its accumulation in the tissue beyond the capacity to be discharged through the natural physiological mechanisms [9].

In conclusion, cobalt could be considered as a risk factor for male reproductive health and therefore men exposed to higher cobalt concentrations on their working places should be carefully monitored. Moreover, it would be beneficial if they are additionally subjected to treatment with drugs such as complex of selenium and vitamins A, C, E which are able to reduce the negative effect of cobalt [9].

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