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HEAVY METALS AFFECTING ANIMAL REPRODUCTION – MICROSCOPIC STUDIES

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Abstract

Pollution of the environment and contamination of animal tissues and organs is serious problem in most countries. In this paper the effects of various environmental factors on structure and function of animal reproductive organs are reported. In ovaries cadmium causes decrease of relative volume of growing follicles and significant increase of the number of atretic follicles. The most frequent ultrastructural alterations observed were undulation of external nuclear membrane, dilatation of perinuclear cistern and endoplasmic reticulum. In a single nickel and nickel–zinc administration experiment on the structure and function of rabbit ovary fine follicular structures were analyzed. Various alterations in the relative volume and follicular antrum formation were found. In testes, the administration of selected environmental contaminants (Hg, Pb, Co, Cd, Ni) resulted in undulation of basal membrane, dilatation of blood vessels in interstitium and occurrence of empty spaces in germinal epithelium. Decreased relative volume of germinal epithelium, increased relative volume of interstitium and increased apoptosis occurrence suggest damaged interstitium and revealed occurrence of oedema as the most significant change. In vitro studies (Hg, Cu) showed dose– as well as time–dependent decrease of spermatozoa motility and cell membrane integrity changes.

Keywords: heavy metals, reproduction, structural alterations, animals

Introduction

Pollution of the environment and contamination of animals including game with cadmium is serious problem in most countries *(Stawarz et al., 2003)*. Increasing concern about pollution of our environment calls for advanced and rapid methods to estimate ecological toxicity *(Nota et al., 2008)*. Xenobiotics, including heavy metals, exist in nature as complex mixtures of compounds with possible interactions *(Stawarz et al., 2009)*. Animal studies on the toxicity of heavy metals have been widely used as model to simulate the impacts of environmental pollution on the human health *(Al–Johany and Haffor, 2009)*. The effects of heavy metals on the health status of pigs *(Lopez–Alonso et al., 2007)*, hens *(Kolesarova et al., 2008, Capcarova et al., 2008)*, brown hares *(Massanyi et al., 2003)*, rabbits *(Roychoudhury and Massanyi, 2008)*, rats *(Massanyi et al., 2007)*, golden hamsters *(Lukac et al., 2007)*, and frogs *(Formicki et al., 2008)*, were examined.

It has been reported that many metals have negative effects on the reproduction in animals *(Blottner et al., 1999, Cigankova et al., 1998)*. In the study of 107 fertile and 103 subfertile male blood and semen specimens the concentrations of calcium, magnesium, zinc, and copper in blood and seminal plasma were not different between the subfertile and fertile group. Weak correlations were demonstrated between blood plasma zinc concentrations and sperm count, sperm motility and abnormal sperm morphology. Zinc concentrations in seminal plasma correlated weakly with sperm count and copper concentrations in blood plasma with motility *(Formicki et al., 2008)*. The zinc deficiency cause degenerative changes in spermatogenic cells after meiosis, their depletion and cumulation in the lumen of seminiferous tubuli. The increased occurrence of malformed spermatids indicates that course of spermatogenesis is impaired. It has been stated that zinc is an indispensable element for a normal course of spermatogenesis *(Cigankova et al., 1997)*. It has been reported that the copper has a toxic effect on the seminiferous epithelium *(Vrzgulova et al., 1993)* In the toxic phase of disease, the germinative epithelium was first damaged. The breaking or decay of the cellular membrane with consequent destruction or cessation of spermatogenesis has been observed and is similar to the alterations found after ischaemia. The toxic effects of copper on seminal plasma are manifested in the decrease of the percentage of motile spermatozoa and in the decrease of malformed sperm cells *(Gamcik et al., 1990)*. Cadmium accumulates mainly in the kidney and liver *(Toman and Massanyi, 1996)*, but it has various effects on male *(Blottner et al., 1999)* as well as female *(Massanyi and Uhrin, 1997)*, reproductive organs. In the study describes the influence of environmental cadmium on testicular proliferation in roe deer the results suggest delayed proliferation during the pre–rutting period in animals with high cadmium exposure, but other indicators of the effects on the testis were not significant *(Blottner et al., 1999)*. The toxic effect of lead on

gonadotropin binding (lower affinity) has been reported in rats *(Combs, 1997)*. With regard to the lead intoxication, hypoplasia of the Leydig cells producing testosterone in atrophic testes was recorded. In the study on male reproductive toxicity of inorganic lead at current European exposure levels have been found an adverse effect of lead on sperm concentration and susceptibility to acid induced denaturation of sperm chromatin *(Bonde et al., 2002)*. The data of the time–course study indicate that the effect of nickel on testosterone production is both time and concentration dependent and not due to cytotoxicity *(Forgacs et al., 2002)*. Generally, it is interesting that elements with oxidation states II (cadmium, copper, lead, mercury, zinc, nickel) all show a strong affinity for ligands such as phosphates, cysteinyl and histidyl side chains of proteins, purines, pteridines, and porhyrins. Hence, all these elements can act at a large number of biochemical sites. All inhibit many enzymes having functional sulfhydryl groups, all bind to and affect the confirmation of nucleic acids, and all disrupt pathways of oxidative phosphorylation, although in each instance the precise response depends upon the individual properties of the metal. On the other hand this study determined high level of zinc in boar semen which might be the protective factor for boar spermatozoa in comparison with other animal species.

Target of this study was to evaluate the role of selected environmental contaminants (heavy metals) on the reproductive parameters in animals and to describe changes that occur on the microscopic level.

Environmental contaminants and ovarian structure

The effects of cadmium on the structure of ovary, oviduct and uterus after an experimental administration were analyzed *(Massanyi and Uhrin, 1996, Massanyi et al., 2007)*. Animals were divided into three groups. In group A rabbits received cadmium i.p. (1.5 mg/kg b.w.) and were killed after 48 h. In another group (C) cadmium was administered p.o. (1.0 mg/kg) for 5 month. The group K was the control. Decreased relative volume of growing follicles and increased stroma after cadmium administration were detected. The number of atretic follicles was significantly higher after administration of Cd.

The most frequent ultrastructural alterations observed were undulation of external nuclear membrane, dilatation of perinuclear cistern and endoplasmic reticulum. In all studied types of cells mitochondria with altered structure were found. In the oviduct the highest amount of epithelium in the group with long–term cadmium administration was found. Microscopic analysis showed oedematization of the oviduct tissue, caused by disintegration of the capillary wall. An electron microscopic analysis showed dilatation of perinuclear cistern. The intercellular spaces were enlarged and junctions between

cells were affected. Mainly after a long–term cadmium administration nuclear chromatin disintegration was present. In the uterus a significant change was determined in the relative volume of glandular epithelium. Increase of stroma volume was a sign of uterus oedamatization caused by damage in the wall of blood vessels and subsequent diapedesis. After cadmium administration alteration in uterus were less expressed, in comparison with ovary and oviduct. Alteration of nuclear chromatin contain following cadmium administration suggests degenerative functional changes.

In another study the effect of cadmium, cadmium+selenium and cadmium+zinc administration on the ovarian structure in Japanese quails was studied *(Nad et al., 2007)*. The morphometric analysis of the relative volume of primary follicles detected the highest value in control group with a similar value in the group with administration of cadmium with selenium. Lower relative volume is reported in group with cadmium and zinc administration and the group with simple cadmium administration ($p \leq 0.05$). The relative volume of growing follicles was very similar in all studied groups (11.33–15.35%), and the relative volume of stroma was very stable (82.59–86.45%). In the evaluation of the number of follicles undergoing atresia detected significantly higher number of atretic primary follicles as well as atretic growing follicles in the group with cadmium administration and cadmium with selenium administration in comparison with control group. In comparison of normal and atretic follicles we report the most negative effect of single cadmium administration on ovarian structure. Selenium co–administration shows protective effects but only the co–administration with zinc prevents significant cadmium ovarian alterations.

In another the effect of nickel on the structure and function of rabbit ovary, with the detection of fine follicular structural changes was analyzed. In the experiment broiler California line rabbits with the weight 3.5–4.0 kg, 4 month old were used. Experimental animals were adult and clinically healthy. Only the females were included in this experiment and divided into groups – experimental groups (P1, P2) and control (K). P1 group received 17.5 g $NiCl_2$.100 kg^{-1} feed mixture and group P2 35 g $NiCl_2$.100 kg^{-1} feed mixture. The feed mixture was served for 100 days.

Environmental contaminants and testicular structure

The effects of mercury administration on the testicular structure of adult rats were evaluated[27]. Rats received mercury ($HgCl_2$) in single intraperitoneal dose 20 mg $HgCl_2$ (group A), 10 mg $HgCl_2$ (group B) and 5 mg $HgCl_2$ (group C) per kilogram of body weight and were killed after 48 hours following mercury administration. After the preparation of histological samples the results were compared with control group (K). In testis undulation of basal membrane, dilatation of blood vessels in

interstitium and occurrence of empty spaces in germinal epithelium were observed. Decreased relative volume of germinal epithelium, increased relative volume of interstitium and increased apoptosis occurrence suggest damaged interstitium and revealed occurrence of edemas. The relative volume of seminiferous tubules showed higher luminization. The number of nuclei was decreased in all experimental groups, what is in positive relation with occurrence of empty spaces. Also other evaluated criteria demonstrated significant differences between control group and experimental groups. This study reports a negative effect of mercury on the structure and function of testes.

The purpose of another study was to assess the effects of lead administration on the testicular structure of adult rats *(Massanyi et al., 2007)*. Rats received lead ($PbNO_3$) in single intraperitoneal dose 50 mg/kg (group A), 25 mg/kg (group B) and 12.5 mg (group C) per kilogram of body weight and were killed 48 h following lead administration. After the preparation of histological samples the results were compared with the control. In testes, dilatation of blood capillaries in interstitium, undulation of basal membrane and occurrence of empty spaces in seminiferous epithelium were detected. An apoptosis assay confirmed increased incidence of apoptosis in the spermatogenetic cells after the lead administration. Also further morphometric analysis showed significant differences in evaluated parameters between control and treated groups. The number of cell nuclei was decreased in lead–treated groups, which is concerned with the occurrence of empty spaces as well as with the higher apoptosis incidence in germinal epithelium.

The effect of cobalt on the testicular structure of adult golden hamsters was analyzed *(Lukac et al., 2007)*. Hamsters in group A received cobalt ($CoCl_2$) in single intraperitoneal dose 20 mg/kg, in group B 10 mg/kg and in group C 5 mg $CoCl_2$/kg body weight and were killed forty eight hours after cobalt administration. After a preparation of histological samples the results were compared with the control. After a cobalt administration dilatation of blood capillaries in interstitium, undulation of basal membrane and occurrence of empty spaces in seminiferous epithelium were detected. Morphometric analysis showed that in all cobalt–treated groups the relative volume of seminiferous epithelium was significantly decreased. In the relative volume of interstitium a significant increase was found between control group and experimental groups. After cobalt administration we have found linear non–significant decrease. Evaluation of diameter seminiferous tubules found increase of this parameter in the all experimental group in comparison with the control. The height of seminiferous epithelium was relatively constant and in all groups but the difference between control and group A was significant ($p \leq 0.05$). Analysis of the lumen diameter of seminiferous tubules detected significantly increase mainly in group B. Evaluation of the number of cell nuclei per a constant area detected an increase of this parameter in experimental group. Results of this study report a negative effect of cobalt on structure and function of testes. All the data

report negative effect of environmental contaminant on the function of testicular structures and are in correspondence with previous reports *(Mathur et al., 2010).*

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