

THE EFFECTS OF CADMIUM ON THE OVARIAN CELL ULTRASTRUCTURE IN VIVO

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Cadmium is an environmental risk factor having various toxic effects both in animals and in humans. Some cadmium has been found in all natural materials that have been analyzed. In the past, chronic effects due to long-term inhalation of cadmium-containing dust were frequently observed. The type and intensity of symptoms depend on individual disposition as well as on intensity and duration of exposure. Cadmium also affects reproductive organs [1–3]. Its action may be either direct, affecting the gonads and accessory organs, or indirect via interference with the hypothalamus–pituitary–gonadal axis [4].

The aim of this study was to determine effects of cadmium on the ultrastructure of various ovarian cells after an experimental administration. The structure of ovarian cells (granulosa, thecal, stromal, endothelial cells) was analyzed by transmission electron microscopy (TEM). In vivo – animals (24) were divided into three groups (K, A, C). In group A (n=8) rabbits received cadmium i.p. (1.5 mg/kg body weight) and subsequently were killed 48 h after administration of cadmium. In group C (n=8) cadmium was administered at a dose of 1.0 mg/kg b.w. for 5 month in pelletized food. The last group (K) was the control, receiving no cadmium. In vitro ultrastructure of granulosa cells was studied after 48 h of culture with (0.2, 10 and 20 ng CdCl₂/ml) or without cadmium.

In vivo study: The qualitative analysis of granulosa cells showed undulation of nuclear membrane, dilatation of perinuclear cistern and endoplasmic reticulum. In theca cells dilatation of endoplasmic reticulum was the most characteristic alteration. Also dilatation of perinuclear cistern was evident. In stromal ovarian cells very intensive dilatation of perinuclear cistern and structures with smooth membranes were detected. In endothelial cells dilated mitochondria with altered inner structure, mainly missing cristae were found. Quantitative analysis of granulosa cells found significant ($p<0.05$) decrease of relative volume of mitochondria in group C in comparison with group A. In ovarian thecal cells a significant ($p<0.001$) increase of the relative volume of endoplasmic reticulum in group A in comparison with control group was detected. In ovarian stromal cells a significant increase of the relative volume of smooth membranes in comparison with control animals was found. In endothelial cells we have observed significantly higher amount of mitochondria and cytoplasm in group A in comparison with control group.

In vitro study: After cadmium administration cell membranes were disintegrated. It was manifested by occurrence of vacuoles in cytoplasm. The vacuoles contained fibrillar or membranous material. The Golgi complex rarely remained intact. Increased number of lysosomes was detected. With increasing cadmium concentrations the number of lipid droplets increased. In some cells the changes were less evident and dense mitochondria with distinct membranes were found. In other cell types the amount of mitochondrial matrix increased and that of membranes decreased. Some mitochondria fused with lysosomes. The endoplasmic reticulum rarely remained intact, and its dilatation was well visible on transverse sections. Nuclei with distinct heterochromatin at the nuclear membrane were often observed. Less frequently nuclei with condensed chromatin reminiscent of pyknosis were observed. Some nuclei had dispersed fine granular chromatin. In other cells changes were less evident, and comprised condensed chromatin in the central part of nuclei. These structural changes of granulosa cells exposed to cadmium were related to premature luteinization of these cells.

Results of our study prove negative effects of cadmium on the ovarian structure also on the ultrastructural level. In previous study [1] we have reported that on microscopic level (in the same animals) with regard to the number of follicles, the lowest number of primary follicles was found after i.p. administration of cadmium. Percentual content of growing follicles was significantly higher and of stroma significantly lower in the control group in comparison with all experimental groups receiving an administration of cadmium. The stimulatory and inhibitory effects of cadmium on progesterone synthesis were recently investigated using the steroidogenically stable JC-410 porcine granulosa cells line, which was genetically modified with gene constructs containing the promoter region of the

cytochrome P450 side chain cleavage gene linked to a luciferase reporter gene [5]. Generally there are few data describing the effect of cadmium on granulosa cells [6] with mainly monitoring and biochemical aspects of toxicity.

Our results describe fine structural alterations of ovarian cells after an administration of cadmium. The negative effect of this common environmental toxicant was detected in all studied cell types and we can conclude that it is cell-dependent.

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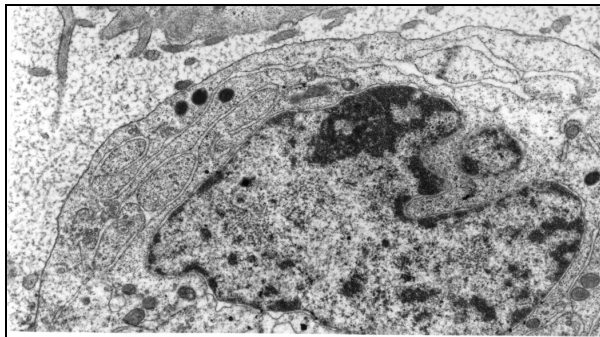


Figure A: In granulosa cells undulation of nuclear membrane enclosing nucleus and dilatation of perinuclear cisterna. Endoplasmic reticulum was dilated (x7.200)

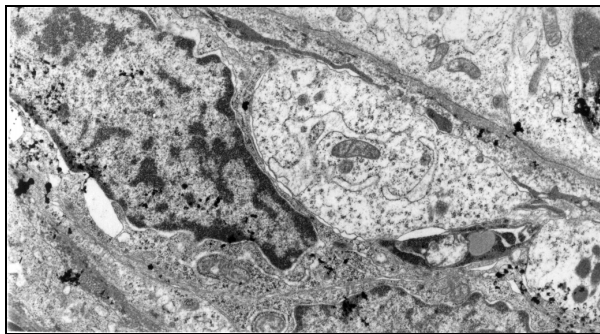


Figure B: In theca cells dilatation of endoplasmic reticulum is the most characteristic sign of cadmium related changes. Also perinuclear cisterna was dilated (x7.200)

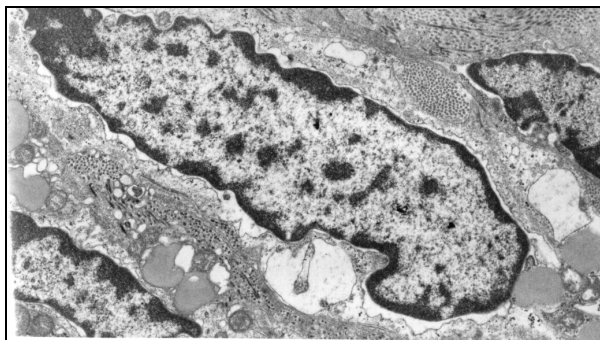


Figure C: Intensive alterations of nuclear membrane with dilatation of perinuclear cisterna and dilated structures with smooth membranes – vacuolization – typical sign of toxic effect (x7.200)