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FEMALE REPRODUCTIVE TOXICOLOGY OF CADMIUM*

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The aim of this study was to determine effects of Cd on the structure of ovary, oviduct and uterus after an experimental administration. Animals were divided into three groups. In group A rabbits received cadmium i.p. and were killed after 48 h. In group C Cd was administered p.o. for 5 month. The group K was the control. Decreased relative volume of growing follicles and increased stroma after Cd 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 Cd 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 was a sign of uterus oedamatization caused by damage in the wall of blood vessels and subsequent diapedesis. After Cd administration alteration in uterus were less expressed, in comparison with ovary and oviduct. Alteration of nuclear chromatin contain following Cd administration suggests degenerative functional changes.

Keywords: Cadmium – ovary – oviduct – uterus – toxicology

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INTRODUCTION

Cd is chemically similar to zinc and occurs naturally with zinc and lead in sulfide ores. Some Cd has been found in all natural materials that have been analyzed. High concentrations in air, water and soil are, however commonly associated with industrial emission sources, particularly non-ferrous mining and metal refining [7].

In the past, chronic effects due to long-term inhalation of Cd-containing dust were frequently observed. The type and intensity of symptoms depend on individual disposition as well as on intensity and duration of exposure. Long-term ingestion of large amounts of Cd has, until now, only been observed in Japan. This has led to kidney dysfunction, as in industrial exposure, and to severe bone disease known as Itai-itai disease [19]. Predominant storage in soft tissue (primary liver and kidney) rather than bone has been reported [1, 2, 4, 14, 15, 16, 21, 25].

Cd has a diversity of toxic effects, including nephrotoxicity, carcinogenity, teratogenicity, endocrine and immune toxicities. Although Cd is not essential for growth and development in mammals, it generally follows the metabolic pathways of essential elements zinc and copper [3, 8, 12, 13, 27].

Cd also affects reproductive organs [5, 6, 11, 28]. Its action may be either direct, affecting the gonads and accessory organs, or indirect via interference with the hypothalamus – pituitary – gonadal axis [22]. Cd chloride administered s.c. induced profound cellular and vascular changes in the ovary of prepubertal rats. The large and medium-size follicles underwent immediate mass atresia and the smaller ones had the same fate after a brief period of resistence [10].

The aim of this study was to determine the histological and ultrastuctural alterations of ovary, oviduct and uterus after the experimental (i.p. and p.o.) administration of Cd.

MATERIAL AND METHODS

All experiments were conducted on rabbits (Hyla, Slovak Center for Agricultural Research, Nitra, Slovak Republic). Animals ($n = 24$) were divided into three groups (K, A, C). Eight rabbits received Cd i.p. (1.5 mg/kg body weight). These animals (group A – acute effects) were killed 48 h after administration of Cd ($CdCl_2$, Sigma, St. Louis, MO, USA). Cd was diluted with a physiological solution to the appropriate concentration. A chronic experiment (group C – chronic effects) was carried out on the same number of animals. In this group Cd was administered at a dose of 1.0 mg/kg b.w. for 5 month in pelletized food. Food and water were available for all animals *ad libitum.* The last group (K) was the control, receiving no Cd.

For histology ovary, oviduct and uterus were fixed in 10% formalin, dehydrated in a graded series of ethanol and embedded in paraffin wax. Whole samples were sectioned on a microtome. The serial sections were stained with haematoxylin and eosin. From photographs based on micromorphological criteria [31, 32] – the number of follicles (primary, < 2 layers of granulosa cells, > 2 layers of granulosa cells, antral

follicles) per constant area, as well as the diameter of follicles and relative volume of follicles and stroma in ovaries, relative volume of surface epithelium, stroma and muscular layer in oviduct and the relative volume of endometrium and myometrium and subsequently the relative volume of surface epithelium, glandular epithelium and stroma in endometrium were evaluated with respect to each organ.

For transmission electron microscopy the samples were fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde diluted in 0.2 M phosphate buffer at 4 °C for 2 h, postfixed with OsO_4, dehydrated in a graded series of ethanol solution, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and observed using JEM – 100 CX-II (JEOL, Japan). From each experiment 20 electron microscopy sections from at least three different replicates were examined.

Analysis of variance and *t*-test were used to calculate basic statistic characteristics and to determine significant differences in structures.

RESULTS

Ovary

In the evaluation of the proportional content of follicles and stroma, it was found that there is significantly higher relative volume of growing follicles (30.71%) and lower stroma (66.27%) in the control group in comparison with both experimental groups receiving Cd (Table 1).

Evaluating the number of follicles, it was found that the highest number of primary follicles was present in the group with the chronic administration of Cd (2.84). After i.p. administration was the level lower (1.33 per 10,000 μm^2). The number of

Table 1
Morphometric parameters in ovary – relative volume of follicles and stroma and average number of follicles (per 10.000 μm^2 and visual field#)

Group	K x ± s	A x ± s	C x ± s
Primary follicles	3.02 ± 2.26	1.55 ± 1.20	2.77 ± 2.16
Growing follicles	30.71 ± 24.16*	5.42 ± 8.23	9.69 ± 8.62
Stroma	66.27 ± 23.94*	93.03 ± 7.98	87.54 ± 9.37
Primary follicles	2.76 ± 1.87	1.33 ± 0.94	2.84 ± 2.20
< 2 layers of GC	0.22 ± 0.22	0.10 ± 0.17	0.38 ± 0.44
> 2 layers of GC	0.13 ± 0.26	0.11 ± 0.26	0.28 ± 0.42
Antral formation	0.13 ± 0.24	0.01 ± 0.06	0.07 ± 0.20
Antral follicles#	0.56 ± 0.78	0.38 ± 0.62	0.79 ± 1.01
Atretic follicles#	0.44 ± 0.70*	2.69 ± 1.78	2.45 ± 1.68

* $p < 0.05$

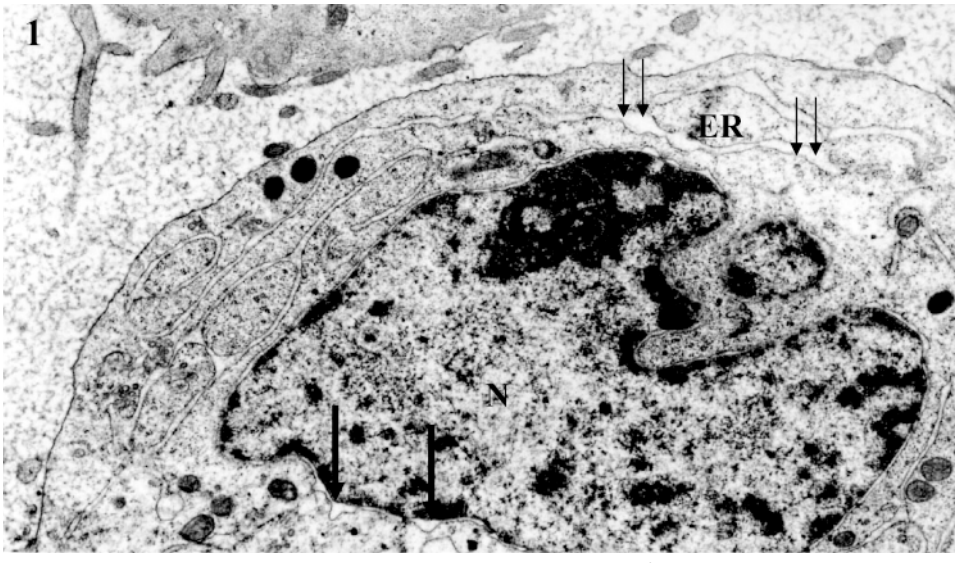

Fig. 1. In ovarian granolosa cells undulation of nuclear membrane (↓) enclosing the nucleus (N) and dilatation of perinuclear cistern was found. Endoplasmic reticulum (ER) was dilated (↓↓) (TEM, ×7200)

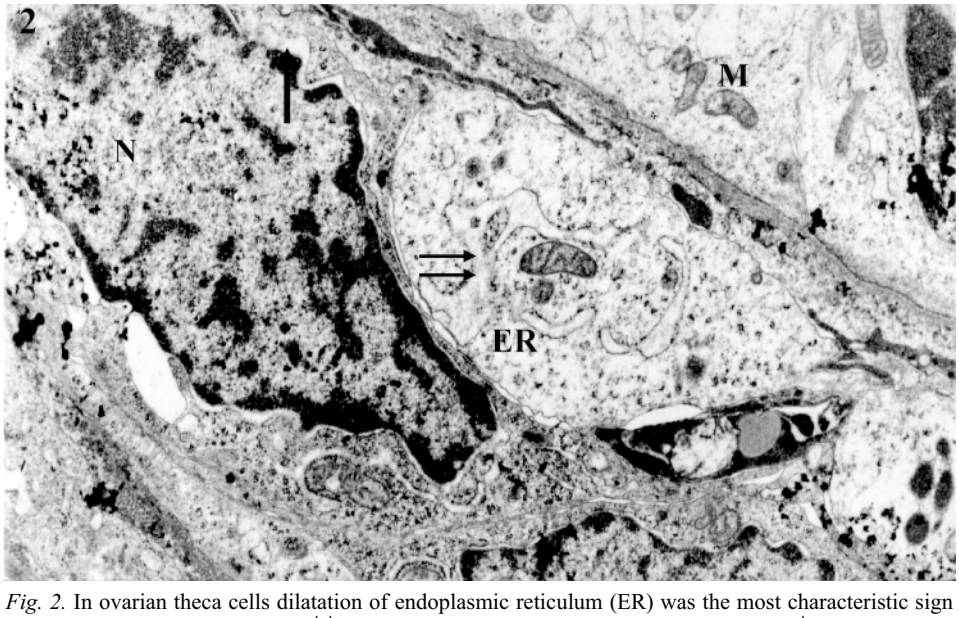

Fig. 2. In ovarian theca cells dilatation of endoplasmic reticulum (ER) was the most characteristic sign of cadmium related alterations (↓↓). Dilatation of perinuclear cistern was evident (↓). Nucleus (N), mitochondria (M) (TEM, ×7200)

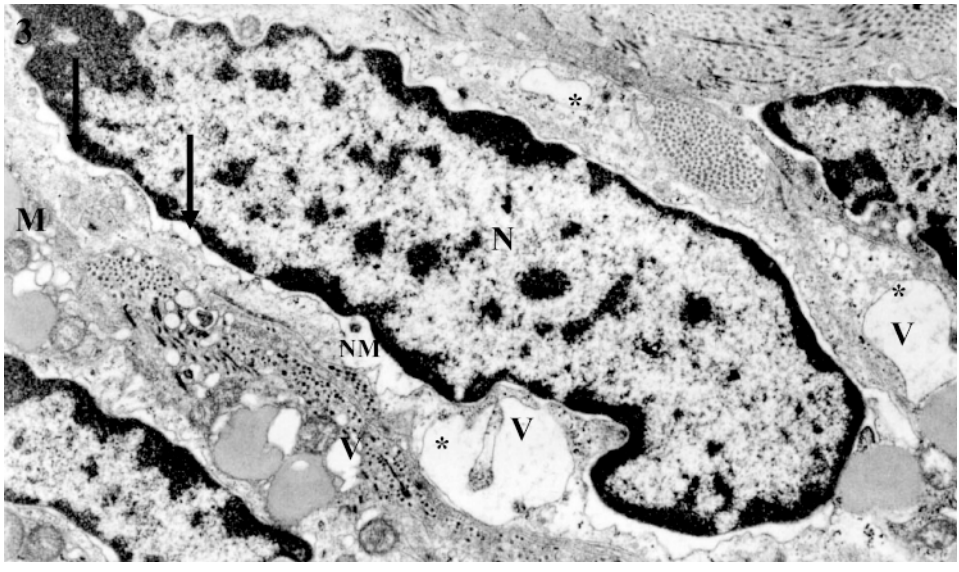

Fig. 3. Intensive alterations of nuclear membrane (NM) with dilatation of perinuclear cistern (↓) and dilated structures with smooth membranes (*) – vacuolisation (V) is a specific sign of cadmium toxicity in ovarian stromal cells. Nucleus (N), mitochondria (M) (TEM, ×7200)

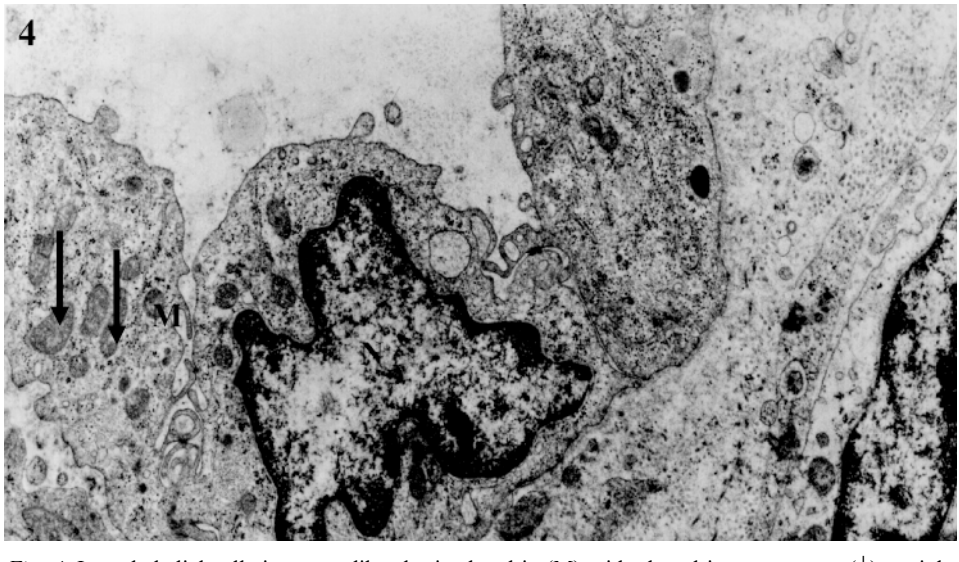

Fig. 4. In endothelial cells in ovary dilated mitochondria (M) with altered inner structure (↓), mainly missing cristae were found after cadmium administration. Nucleus (N), mitochondria (M) (TEM, ×7200)

atretic follicles was significantly ($P < 0.05$) higher in both groups with experimental administration of Cd.

Electron microscopic analysis showed that in experimental groups most frequent alterations are undulation of external nuclear membrane, dilatation of perinuclear cistern and endoplasmic reticulum (Fig. 1). In theca cells dilatation of endoplasmic reticulum was the most characteristic sign of alterations. Also dilatation of perinuclear cistern was evident (Fig. 2). In stromal ovarian cells very intensive alterations of nuclear membrane and dilated structures with smooth membranes were detected (Fig. 3). In endothelial cells dilated mitochondria with altered inner structure, mainly missing cristae were found (Fig. 4). In all studied types of cells mitochondria with altered structure were observed.

Oviduct

In evaluating the relative volume of epithelium, stroma and muscular layer in oviduct we found that the highest amount of epithelium was in the group with long-term Cd administration. In the other groups the relative volume was very similar without significant differences (Table 2).

Microscopic analysis showed oedematization of the oviduct tissue, caused by disintegration of the capillary wall, after a high level of Cd intake. Dense material on the top of well-vascularized capillary mucosa was evident after Cd administration. The amount of muscular layer was in both experimental groups lower, but the differences were not significant.

An electron microscopic analysis showed that in the oviduct of control rabbits two kinds of epithelial cells, namely cylindrical cells with cilia and glandular cells were present. After a Cd administration dilatation of perinuclear cistern (between internal and external nuclear membrane) occurs (Fig. 5). Nuclear membrane showed signs of undulation. Also a dilatation of granular endoplasmic cisternae was detected (Fig. 5). The intercellular spaces were enlarged (Fig. 6) and the junction between cells were affected. In both cell types vacuolization of cytoplasm as a typical sign of degeneration was detected (Fig. 6). First of all after a long-term Cd administration nuclear alterations in form of chromatin disintegration were present (Fig. 7), indicating general cellular damage.

Table 2
Morphometric parameters in oviduct – relative volume of surface epithelium, stroma and muscular layer

Group	K x ± s	A x ± s	C x ± s
Surface epithelium	23.12 ± 7.30	22.11 ± 5.57	25.50 ± 6.52
Stroma	19.11 ± 5.02	24.96 ± 5.33*	19.89 ± 6.09
Muscular layer	57.77 ± 11.32	53.93 ± 6.61	54.61 ± 8.80

* $p < 0.05$

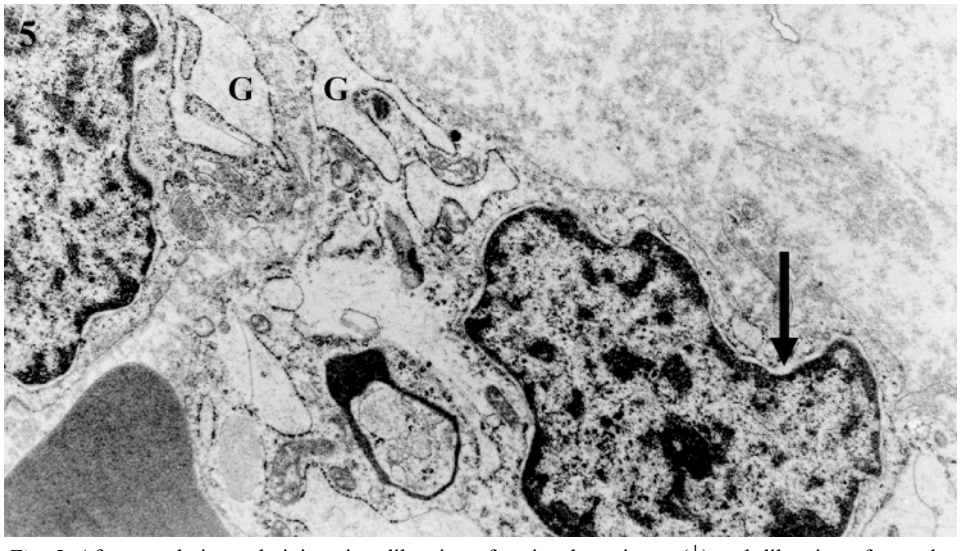

Fig. 5. After a cadmium administration dilatation of perinuclear cistern (↓) and dilatation of granular endoplasmic reticulum (G) are the most significant changes in oviduct (TEM, ×7200)

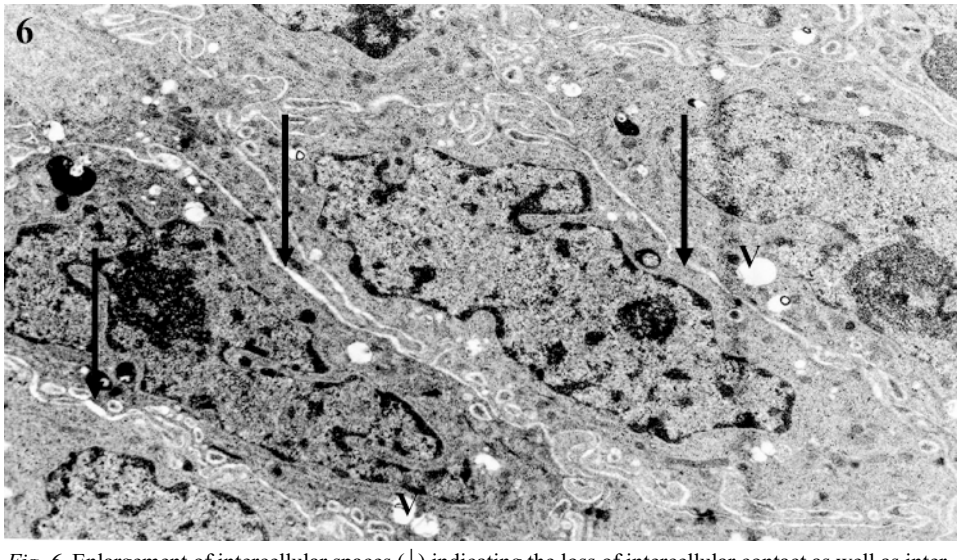

Fig. 6. Enlargement of intercellular spaces (↓) indicating the loss of intercellular contact as well as intercellular communication and vacuolization (V) of cytoplasm are also significant alteration of cellular alterations in oviduct (TEM, ×7200)

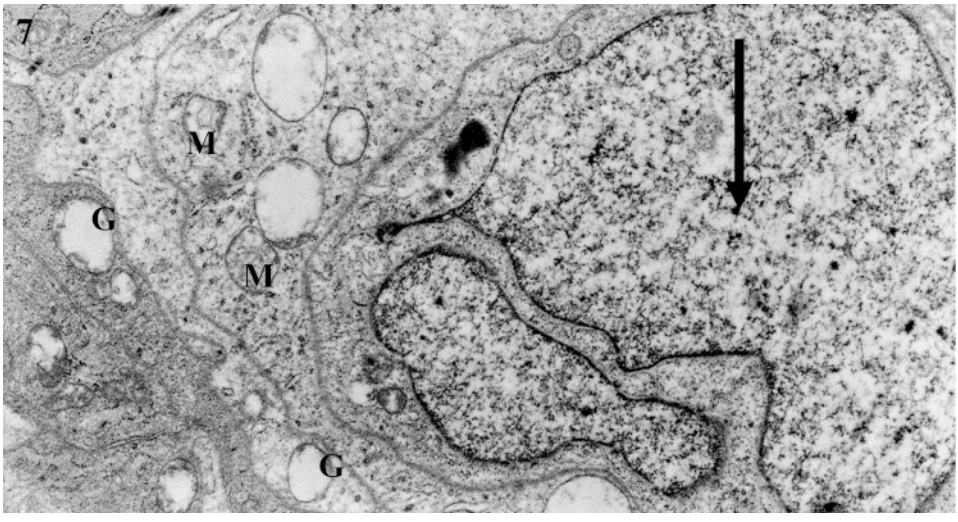

Fig. 7. Particularly after a long-term cadmium administration disintegration of nuclear chromatin (↓), dilated endoplasmic reticulum (G) and mitochondrial alterations (M) were detected in oviduct (TEM, ×7200)

Uterus

In the uterus the relative volume of endometrium and myometrium was in the range of 48.42 – 50.17% and of 49.83 – 51.58%, respectively (Table 3). In the study of relative volume of surface epithelium, glandular epithelium and stroma of uterus, a significant difference was determined in the relative volume of glandular epithelium in group A in comparison with group C ($P < 0.05$) what suggest a toxic cellular effect of short-term Cd administration. An increase in the relative volume of stroma is a

Table 3
Morphometric parameters in uterus – relative volume of endometrium and myometrium and relative volume of surface epithelium, glandular epithelium and stroma in endometrium

Group	K x ± s	A x ± s	C x ± s
Endometrium	48.73 ± 14.25	50.17 ± 9.41	48.42 ± 7.95
Myometrium	51.27 ± 14.25	49.83 ± 9.41	51.58 ± 7.95
Surface epithelium	5.87 ± 1.84	5.47 ± 1.09	6.99 ± 3.15
Glandular epithelium	5.78 ± 2.43	4.37 ± 1.87	8.07 ± 3.41*
Stroma	88.35 ± 2.98	90.15 ± 2.33	84.94 ± 3.55

* $p < 0.05$ [A–C]

sign of uterus oedamatization caused by damage in the wall of blood vessels and subsequent diapedesis.

An electron microscopic study detected in uterus endometrium cells of surface epithelium – tall luminal cells lying on basal membrane. Glandular cells are very similar to the cells of surface epithelium and in middle and profound layers are of one layer. The cells of surface epithelium have kinocilia. Blood capillaries are under the basal membrane lined with endothelial cells. After Cd administration in uterus are less expressive in comparison with ovary and oviduct. Any alterations of the endothelium of blood vessels were found (Fig. 8). After a long-term Cd administration visible alteration of nuclear chromatin (disintegration) are reported suggesting degenerative functional changes (Fig. 9).

It can be concluded that experimental administration of Cd affects mainly the structure of various ovarian cells and the effect of this common environmental toxicant is specific causing primarily undulation of nuclear membrane, nuclear chromatin redistribution, and finally dilatation of perinuclear cistern and endoplasmic reticulum

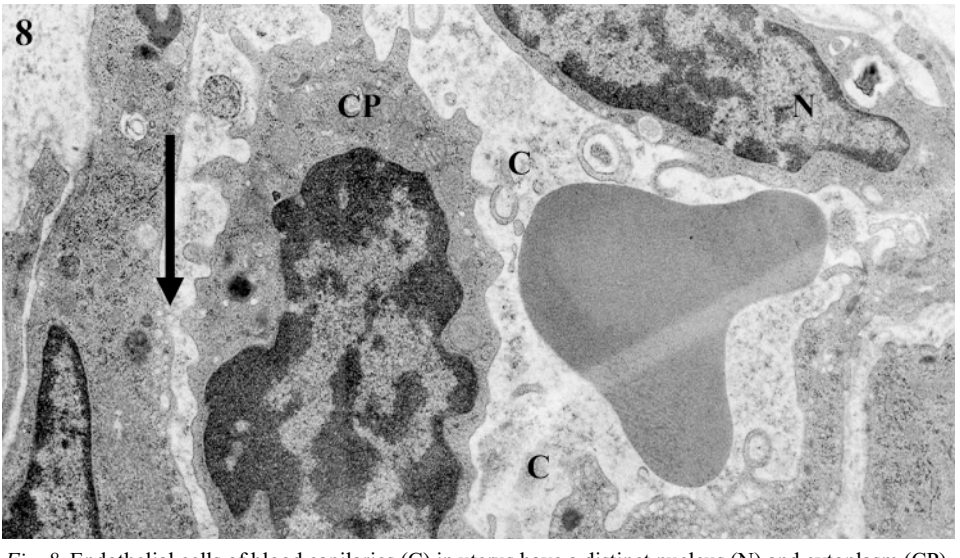

Fig. 8. Endothelial cells of blood capilaries (C) in uterus have a distinct nucleus (N) and cytoplasm (CP). Typical are pinocytosis vesicles (↓) (TEM, ×7200)

DISCUSSION

Results of our study prove negative effects of Cd on the structure of female reproductive organs also on the ultrastructural level. In *in vitro* cultured porcine ovarian granulosa cells similar alterations were reported [18]. Cell membranes were disinte-

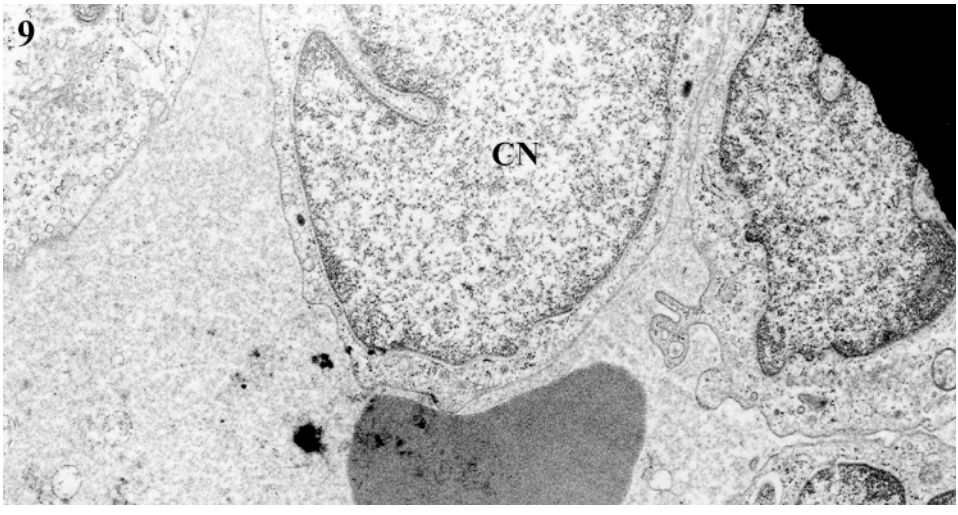

Fig. 9. Endothelial cells of blood capilaries (C) in uterus have a distinct nucleus (N) and cytoplasm (CP). Typical are pinocytosis vesicles (↓) (TEM, ×7200)

grated manifested by the occurrence of vacuoles in the cytoplasm. The vacuoles contained fibrillar or membranous material. The Golgi complex rarely remained intact. Increased number of lysosomes was detected. The stimulatory and inhibitory effects of Cd 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, the effects of Cd on reproductive parameters of various animal species are incompletely described. Most of studies describe accumulation of this toxic element in ovaries [27, 29, 30]. On the other hand, ultrastructural observation and specially quantification of toxic effect of cellular structures of various cells is inadequate. Our previous results supplement the knowledge that Cd has a dual action in stable porcine granulosa cells as low concentrations activate, whereas high concentrations inhibit progesterone synthesis [18].

Exposure of human granulosa cells to Cd resulted in morphological alterations in the monolayer depending on dose with longer exposure, cells began to separate from each other by contacting towards the centre and assuming a circular shape [22].

Generally, there are few data describing the effect of Cd on granulosa cells [5, 9, 26] mostly monitoring the biochemical aspects of toxicity. In luteal cells only the interference of Cd with steroid biosynthesis in rat luteal cells *in vitro* was studied [23]. In relation to stromal cells in ovary many reports were published. Myeloid and erythroid hematopoietic progenitors and stromal stem cells as possible targets were studied [29]. The *in vitro* assays showed that various hematotoxic compounds exert different effects on these cell populations. *In vitro* exposure of murine bone marrow

cells to Cd indicated that hematopoietic or stromal bone marrow cells were targets. Stromal cells were more affected compared to myeloid cells. The relation of Cd to vascular disorders such as atherosclerosis in experimental animals was studied [10]. Cd destroys the monolayer of endothelial cells and the cytotoxicity is protected by zinc and copper without metallothionein induction. Endotelial alteration of Cd are described mainly in liver, causing an inflammatory processes that plays a major role in the secondary injury of the liver, and infiltration of neutrophils at the site of necrosis is a common observation [20].

In relation to oviduct there is a serious lack of information. It has been reported that in relation to all reproductive organs Cd mainly accumulates in oviduct [17]. Also our finding report more intensive alterations mainly on ultrastructural level in comparison with uterus.

Regarding the uterus structure, the most interesting finding is the endometrium oedamatization caused by damage in the wall of blood vessels and subsequent diapedesis. In a group of female rats exposed to Cd a dose-dependent and time-related increase of the media as well as signs of perivascular inflammatory reaction was reported with utrastructurally apparent injury to the microcirculation in uterus [24].

In general, it can be concluded that our results clearly confirm that even there is relatively high Cd accumulation in kidneys and liver reproductive organs are very sensitive to Cd exposure mainly due to high mitotic activity in ovaries (granulosa cells) and uterus (endometrium) causing structural disorders earlier that in predominant organs. These alterations are mainly, present at the cellular level, expressing general cellular functional alterations resulting in restricted organ function.

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