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

Acta morphologica et anthropologica, 25 (1-2)
Sofia ● 2018

Impact of Cadmium on Male Fertility

Ekaterina Pavlova[*]*, Nina Atanassova*

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

* Corresponding author e-mail: e_bankova@yahoo.com

Cadmium (Cd) is a heavy metal and a major environmental pollutant. The general population is exposed to Cd mainly via drinking water and food products. It accumulates and is proved to cause severe damage to a variety of organs such as lung, brain, testis, kidney, liver, blood system and bone. Cd exerts direct cytotoxicity within the testis, mainly targeting two specific cell populations, the Sertoli cells and the Leydig cells, with consequent impairment of spermatogenesis and endocrine function. Cd induced oxidative stress in somatic and germ cells, mainly mediated by mimicry and interference with essential ions, beyond apoptosis occurring in germ cells. After Cd treatment disturbance of the hypothalamus-pituitary-gonadal axis is also reported.

Key words: cadmium, testis, fertility

Cadmium, in its pure form, is a soft silver-white metal, which is present in the earth crust in association with multiple different metals. Cd is indeed extracted as a secondary product, during the processing of different metals, such as zinc (Zn), lead (Pb), or copper (Cu). The presence of Cd and Cd compounds in the environment is a consequence of both natural and anthropic processes. Natural sources of Cd include volcanic activity, weathering consumption of rocks, sea aerosols, forest fires and mobilization from soils and landfills. As a derivative of anthropic activities, Cd and Cd compounds, such as Cd-chloride ($CdCl_2$), Cd-acetate and Cd-carbonate, derive from batteries, pigments, plastic stabilizers, pesticides and fertilizers, and photovoltaic devices, as well as from rubber processing, galvanization process, fossil combustion and waste incineration [6]. For the majority of animal species tested, the absorption of Cd can range from 0.5% to 3.0%, while in humans from 3.0% to 8.0% of the dose administered [20]. Factors influencing the uptake are chemical components of the diet, the body's nutritional status, age and gender proportionally to dosage used and time exposure to Cd [44]. Independently from the dietary Cd uptake, women seem to be more prone to Cd-related health effects, suggesting that a gender difference might exist in the susceptibility to Cd toxicity or in the body burden of Cd, probably because of differences in Cd absorption [13]. Indeed, the gastrointestinal absorption of dietary Cd is influenced by dietary intake of essential nutrients, including iron (Fe), Zn and selenium (Se) [6]. Cd concentration in blood is a marker of both recent and cumulative exposure, whereas urinary concentration mainly mirrors cumulative exposure [6, 13]. Among environmentally exposed population, to-

bacco smokers are the most exposed subjects, since tobacco leaves accumulate large amounts of Cd, making tobacco smoke the main source of Cd in smokers [6]. Non-smokers are exposed to Cd by dietary intake of contaminated food (particularly cereals and grains, leafy vegetables, potatoes and offal) and contaminated water, and vegetarians intake of Cd from food is almost double, compared to non vegetarians [13]. The occupational exposure occurs almost exclusively by inhalation of Cd-polluted fumes or dust and by ingestion through dust-contaminated hands. Workers in the metal refinery industry that release Cd have been shown to suffer from impaired health, such as damaged lungs, diarrhoea, stomach pains and severe vomiting, bone fracture, reproductive failure and possibly even infertility, damage to the central nervous system, psychological disorder, possibly DNA damage or cancer development [47].

It has been suggested that Cd is involved in carcinogenesis in multiple organs including kidney, prostate, liver and pancreas [4]. In fact, the International Agency for Research on Cancer (IARC) [18] classified Cd as a known human carcinogen in 1993 and Cd is ranked the 7th toxicant in the Priority List of Hazardous Substances of the Agency for Toxic Substances and Disease Registry [5, 49]. International and governmental agencies have made efforts to control and lower the Cd exposure to the general public in recent years. Nevertheless, Cd has a long biological half-life (20–40 years in humans) and it accumulates in the body over a considerable period of time. It accumulates and is proved to cause severe damage to a variety of organs such as lung, brain, testis, kidney, liver, blood system and bone [44, 55]. After absorption Cd is transported by blood and stored in organs rich in metallothionein (heart, intestine, kidney, liver, lung, pancreas, spleen and stomach), which exhibits high binding affinity for Cd [53].

Data showed that Cd affects the male reproductive system from embryonic stages to adulthood, and has adverse effects on gonadal development [52]. In mouse embryos, administration of Cd caused reduced genital ridge size and retarded migration of primordial germ cells into the genital ridges, resulting in attenuated populations of germ cells, aberrant maturation of gametes and subfertility [1, 51]. Cd has been demonstrated to affect spermatogenesis and/or semen quality and endocrine function, by different pathogenetic mechanisms. Cd severely affects testis structure, by damaging vascular endothelium and blood-testis barrier (BTB) integrity, and by inducing inflammation and apoptosis within the testis [24, 25]. Moreover, Cd exerts direct cytotoxicity within the testis, mainly targeting two specific cell populations, the Sertoli cells and the Leydig cells. It induced oxidative stress in somatic and germ cells, mainly mediated by mimicry and interference with essential ions, beyond apoptosis occurring in germ cells **(Fig. 1)** [13]. The interference with selected signaling pathways and the interference with the epigenetic regulation of genes involved in the regulation of the reproductive function, have been hypothesized as additional mechanisms of Cd-induced reprotoxicity, but have not been specifically investigated. Lastly, disturbance of the hypothalamus-pituitary-gonadal axis is also reported after Cd treatment.

Cadmium induces oxidative stress damage by decreasing the biological activities of some antioxidants, such as superoxide dismutase and glutathione peroxidase [23, 40, 45] with significant reductions in testicular function and androgen secretion [33]. Oxidative stress induced by Cd was associated with production of reactive oxygen species comprising mainly superoxide radical anion, hydrogen peroxide and hydroxyl radical. Oxidative stress is a common factor in about half of the infertile men, illustrating the importance of Cd [33]. It either leads to oxidative damage or activates signal transduction pathways to initiate defense responses [38]. Exposure to Cd is associated with elevated lipid peroxidation in many organs, inclusive the testis [33]. In birds, Cd affects various structures and metabolic processes, such as nucleic acids, carbohydrates, energy metabolism, protein synthesis and enzyme systems and serum testosterone levels [11].

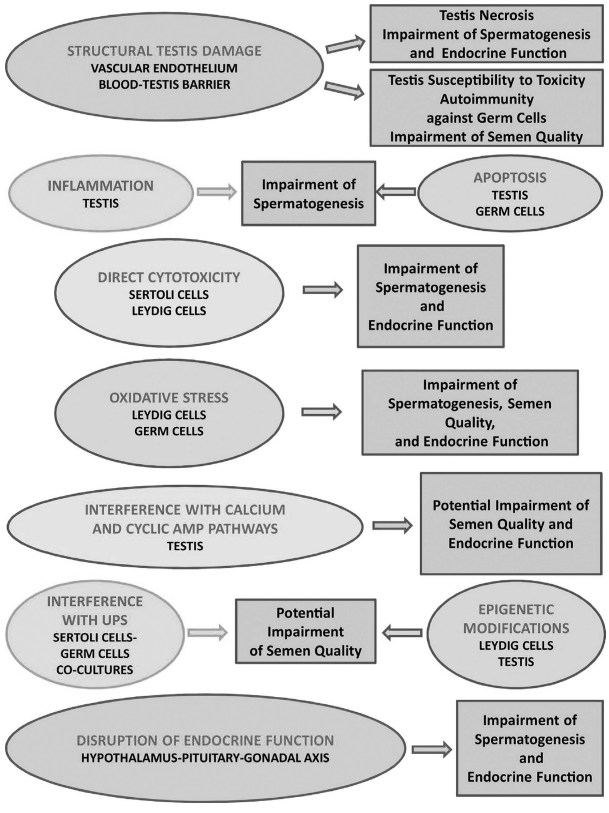

Fig. 1. Schematic overview of the proposed pathogenetic mechanisms of Cd-induced reprotoxicity by de Angelis et al. [2017]

Based on variability of testicular damage, several pathological mechanisms of Cd- induced testicular toxicity have been proposed. In mammals, among the most frequent mechanisms proposed were morphologic alterations and dysfunction in blood vessels [33]. Cadmium causes specific injury to the internal spermatic artery, its testicular and epididymal branches and the pampiniform plexus. It was suggested that Cd in almost all species with scrotal testis, acts principally on the blood vessels of the testis and epididymis, making them more permeable [46], which in turn determines fluid leakage into testis interstitium, followed by edema, hemorrhage, inflammation, hypoxia and, consequently, necrosis of the testis [3]. It was shown that Cd produced this effect by causing the breakdown of the junctions between endothelial cells of testicular capillaries and venules [39]. In particular, Cd was shown to induce alterations in the expression and function of the calcium-dependent cell adhesion molecule as vascular endothelial cadherin (VE-cadherin) at the cell–cell contacts, and a reorganization of the actin cytoskeleton [26, 42]. Cadmium induces a form of programmed necrosis in endothelial cells through disintegration of lysosomes followed by proteolysis, lipidolysis and digestion of nucleic acids resulting in the deterioration of physiological functions [33]. A specific metal ion transporter, ZIP8, has been identified as an enhancer of Cd uptake by vascular endothelial cells in the testis of mice, and its expression has been found to be associated to sensitivity to Cd-induced testis injury [12]. Thus reinforcing

the hypothesis that this transporter might be implicated in the differential susceptibility of animals to Cd toxic effects on vascular endothelial cells. The results of these studies suggest that vascular endothelium damage resulting in necrosis within the testis, may ultimately affect spermatogenesis and testis endocrine function [13].

Vascular smooth muscle cells (VSMC) can perform both contractile and synthetic functions, which are characterised by changes in morphology, proliferation and migration rates, and the expression of different marker proteins [33, 43]. VSMC are involved in physiological functions and pathological changes taking place in the vascular wall. In general, VSMC are sensitive to Cd cytotoxicity without any species-related differences, mainly due to a higher accumulation of the metal within cells [22]. Cadmium blocks the binding of androgen to the receptor but did not alter its affinity, suggesting that the metal is an inhibitor of hormone binding and may play a role in regulating testicular function and male fertility [33, 35].

As opposed to vascular mediated toxicity, it has been postulated that Cd exerts its effects via the physical and chemical properties of the Cd^{+2} ion, namely its similarities to calcium and zinc. As such, Cd is likely to substitute calcium or zinc in crucial physiological processes that are mediated by these ions, resulting in the activation and/or inhibition of several signaling pathways [48]. Zinc has a relationship with many enzymes in the body and can prevent cell damage through activation of the antioxidant defense system [33]. Decreased utilization of zinc by spermatogenic cells due to competitive action of Cd may cause disturbance in sperm developing process [2]. Several experimental studies in animals showed that Cd migh interfere with Se at multiple levels. Se is an essential element with pivotal functions in the maintenance of male reproduction, by influencing structure of the testis, spermatogenesis, semen quality and, ultimately, fertility [13].

An experimental *in vivo* study in animals showed that Cd exposure induced testis inflammation. Cd-loaded rats developed signs of testis inflammation, with significantly increased expression of inflammation markers, including inducible nitricoxide synthase, cyclooxygenase-2, tumor necrosis factor-α, nuclear factor-kB, and heme oxygenase-1, in testis homogenates [15]. Cd-induced testis inflammation resulted in widespread necrosis and vacuolization of the seminiferous epithelium cells, together with interstitial tissue edema and hemorrhage. These pathological changes were associated to an impairment of spermatogenesis [13, 15].

Sertoli cells show the major structural and functional alterations after Cd exposure, even at doses that do not result in visible damage within the testis [13]. Sertoli cells play crucial roles in supporting the self-renewal and differentiation of spermatogonial stem cells into mature sperm [27]. *In vitro* studies suggest that the Sertoli cell is the most vulnerable target of cadmium chloride and they are more sensitive than Leydig cells to Cd- induced damage [33]. The morphological changes in Sertoli cells after Cd exposure are associated with the induction of apoptosis [57]. Cadmium may selectively compromise the development and maintenance of the inter-Sertoli cell tight junctions (TJ), without affecting their secretory activity or the cell number and viability. In mouse, rat and cock Sertoli cells treated with Cd assumed chromatin condensation, nuclear cleaved into dense bodies, lamellar slight endoplasmic reticulum expansion, swelling mitochondria, and pathological vacuoles [7, 8, 31, 33].

The blood-testis-barrier (BTB) is a unique structure, formed by the tight junctions between adjacent Sertoli cells, which bisects the seminiferous epithelium into the basal and the apical/adluminal compartments, by segregating meiotic and post-meiotic germ cells behind the barrier in the apical compartment. Therefore BTB prevents not only the passage of cytotoxic agents from the blood into the seminiferous tubules, but also the passage of antigenic products of germ cell maturation into the circulation, which

might generate autoimmunity against germ cells [9, 13]. Although BTB is not a static ultrastructure, but undergoes massive remodeling during spermatogenesis in order to permit the transit of spermatocytes (preleptotene spermatocytes) [9, 13]. A damage of the BTB is associated with germ cells loss and reduced total sperm count, which determine subfertility or infertility conditions. Cadmium has been shown to dose- dependently affect BTB integrity, by inhibiting the establishment or inducing the disruption of the TJ between rat Sertoli cells *in vitro*, through a down regulation of occludin, a TJ integral membrane protein [10]. Setchell and Waites [46] demonstrated that the BTB is more vulnerable to Cd toxicity than the microvessels in adult rat testes, since the damage to the BTB occurred prior to the microvessels found in the interstitium. In addition, a single low dose of Cd at 1 mg/kg b.w. disrupted TJ associated microfilaments in rat Sertoli cells and also induced spermiation failure without visible vascular lesion in the testes [48]. Other studies have shown that E-cadherin is one of the primary targets of Cd toxicity in epithelial cells, since Cd interacts with the putative calcium-binding motif in E-cadherin, causing a disruption of the cadherin-based cell adhesion. But since E-cadherin coexists with tight junction-proteins (e.g., occludin, claudins, JAM-A) at the BTB, Cd would have immediate access to E-cadherin, making the testis more susceptible to Cd toxicity. Moreover, testosterone counteracted the Cd disruptive effects, possibly by inducing the expression of TJ integral membrane proteins such as occludin. Therefore, testosterone plays a crucial role in the regulation of Sertoli cells TJ-permeability barrier [10], which is consistent with data that androgen promotes the BTB integrity and cell adhesion function in the testis [36, 54]. These observations also illustrate that androgen (or a manipulation of the androgen receptor in Sertoli cells) can be a potential target candidate to manage Cd-induced testicular toxicity.

The studies focused on the correlation between exposure to Cd at environmental concentrations and semen quality are controversial. Several studies found a significant negative correlation between Cd concentration and semen parameters, whereas some studies failed to demonstrate a clear correlation between Cd exposure and semen quality [13]. Many studies in man and in various species of mammals showed that Cd induces various changes in testicular histopathology. A marked reduction of seminiferous tubular diameter after the high dose of Cd, along with the conspicuous decrease of the tubular volume density was reported [14, 33]. Repeated injections of low doses of Cd also impair spermatogenesis. Microscopical changes were observed in the germinal epithelium like necrosis, irreversible degeneration of germ cells and progressive sloughing from the basement membrane [33]. All testicular germ cell populations can be affected by Cd. This includes a decrease in number of spermatogonia and spermatocytes, aberrant morphology in all developing stages, release of immature cells into the lumen [4, 34, 41 58] and failure in spermiation [17]. Elongated and round spermatids, as well as spermatocytes were found in the tubule lumen in > 98% of tubules [37]. Spermatocytes displayed morphological characteristics of apoptosis, including chromatin condensation, cell shrinkage and apoptotic body formation in fowl [31, 33]. Besides sperm concentration, sperm motility is also severely affected by Cd. Sperm motility is recognized to be more sensitive to this trace element, as reduced sperm motility has been observed at a dose far below the dose affecting sperm production. However, it is concluded that Cd accumulation in germinal cells and Cd effects on sperm count and sperm motility are dose- and time dependent [1]. Data by Ige et al. [19] demonstrated that pre-treatment of rat model with Allium cepa extract prevented $CdSO_4$-induced reproductive toxicity by improving sperm quality and enhancing testicular lipid peroxidation status.

In male rodents, it is well established that Cd significantly alters the circulating levels of several hormones (e.g., testosterone, LH, FSH, Inhibin-B) [28, 32]. Previous

studies have shown that Cd impairs the testosterone production in isolated Leydig cells without affecting their viability [30, 56], demonstrating that steroidogenic disruption in Leydig cells is likely to be an initial target of Cd toxicity as an endocrine modulator. Cd can also modify hormone levels by affecting the hypothalamic-pituitary-testicular axis in different aspects, not only via its effects on Leydig cells. It was also shown that, in the testis of mice and rats, Cd affects the expression of steroidogenesis enzymes, such as StAR, cholesterol C20-22 desmolase, 17 β-hydroxylase, 17 β-hydroxysteroid dehydrogenase [21], and suppresses the expression of LH receptor [16, 28]. A large number of studies documented that Cd mimics the function of steroid hormones; therefore, this "metallohormone" has been proposed as endocrine disruptor interfering with endogenous endocrine system. It has been shown that Cd can directly bind to estrogen receptor and androgen receptor, and that Cd exerts strong estrogenic-like and androgenic-like actions, both *in vivo* and *in vitro* [13]. Estrogenic effects of Cd are mediated by the high affinity binding to the ligand-binding domain of estrogen receptor [50], the receptor isoform that drives the mitogenic actions of E_2 in target organs. Cd has been shown to prevent androgens from binding to their receptor, and to mimic the actions of androgens on cell growth and gene expression *in vitro* [35]. Moreover, *in vivo* studies in castrated rats demonstrated that low doses of Cd dose-dependently increased the weight of the prostate gland and of the seminal vesicles and this effect was blocked by an antiandrogen, thus suggesting that Cd actions are mediated by androgen receptor [13, 35]. All of these Cd effects on the endocrine system involved not only direct effects on target organs and cells, but also impairment of the circadian release of noradrenaline, with subsequent changes in GnRH secretion from the hypothalamus, in LH and prolactin secretion from the pituitary, and in testosterone circulating concentrations, in male rats [28, 29]. In summary, the results of these studies suggest that disruption of the hypothalamus-pituitary-gonadal axis may mediate the toxic effect of Cd on spermatogenesis and endocrine function of the testis [13].

Conclusions

Despite the heterogeneity of study designs in animal models and epidemiological studies in man, the data in literature strongly determine cadmium as reprotoxic element. Cadmium directly affect selected cell populations of the testis, which include direct cytotoxicity and functional impairment of Sertoli and Leydig cells, induced oxidative stress in both somatic and germ cells. It affects germ cell populations leading to reduced semen density and quality. Cadmium treatment causes a direct disturbance of the hypothalamus-pituitary-gonadal axis, which might impair spermatogenesis and endocrine function of the testis.

References:

1. **Alaee, S., A. Talaiekhozani, S. Rezaei, K. Alaee, E. Yousefian.** Cadmium and male infertility. - *J. Infertil. Reprod. Biol.,* **2**(2), 2014, 62-69.
2. **Amara, S., H. Abdelmelek, C. Garrel, P. Guiraud, T. Douki, J. L. Ravanat, A. Favier, M. Sakly, K. Ben Rhouma.** Preventive effect of zinc against cadmium-induced oxidative stress in the rat testis. - *J. Reprod. Dev.,* **54,** 2008, 129-134.
3. **Aoki, A., A. P. Hoffer.** Reexamination of the lesions in rat testis caused by cadmium. - *Biol. Reprod.,* **18** (4), 1978, 579–591.
4. **Aoyagi, T., H. Ishikawa, K. Miyaji, K. Hayakawa, M. Hata.** Cadmium-induced testicular damage in a rat model of subchronic intoxication. - *Reprod. Med. Biol.,* **12,** 2002, 59-63.

5. **ATSDR.** The 2007 CERCLA Priority List of Hazardous Substances. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, GA, 2007.
6. **ATSDR.** Toxicological profile for cadmium. *In: Toxicological Profiles, Agency for Toxic Substances and Disease Registry,* 2012.
7. **Bekheet, S. H. M.** Cadmium chloride rapidly alters both BTB tight junction proteins and germ cells in young rat testes. - *Egypt. Acad. J. Biolog. Sci.,* **2,** 2010, 59-74.
8. **Bizarro, P., S. Acevedo, G. Nino-Cabrera, P. Mussali-Galante, F. Pasos, M. R. Avila-Costa, T. I. Fortoul.** Ultrastructural modifications in the mitochondrion of mouse Sertoli cells after inhalation of lead, cadmium or lead-cadmium mixture. - *Reprod. Toxicol.,* **17,** 2003, 561-566.
9. **Cheng, C. Y., D. D. Mruk.** The blood-testis barrier and its implications for male contraception. – *Pharmacol. Rev.,* **64**(1), 2012, 16-64.
10. **Chung, N. P., C. Y. Cheng.** Is cadmium chloride-induced inter-sertoli tight junction permeability barrier disruption a suitable in vitro model to study the events of junction disassembly during spermatogenesis in the rat testis? - *Endocrinology,* **142**(5), 2001, 1878-1888.
11. **Cinar, M.** Cadmium and effects at biological system. – *Veterinarium,* **14,** 2003, 79-84.
12. **Dalton,T. P., L. He, B. Wang, M. L. Miller, L. Jin, K. F. Stringer, X. Chang, C. S. Baxter, D. W. Nebert.** Identification of mouse SLC39A8 as the transporter responsible for cadmium-induced toxicity in the testis. - *Proc. Natl. Acad. Sci.U. S. A.,* **102**(9), 2005, 3401-3406.
13. **De Angelis, C., M. Galdiero, C. Pivonello, C. Salzano, D. Gianfrilli, P. Piscitelli, A. Lenzi, A. Colao, R. Pivonello.** The Environment and Male Reproduction: the Effect of Cadmium Exposure on Reproductive System and Semen Quality and its Implication in Fertility. - *Reprod. Toxicol.,* **73,** 2017, 105-127
14. **De Souza Predes, F., M. A. Diamante, H. Dolder.** Testis response to low doses of cadmium in Wistar rats. - *Int. J. Exp. Pathol.,* **91,** 2010, 125-131.
15. **Fouad, A. A., W. H. Albuali, I. Jresat.** Simvastatin treatment ameliorates injury of rat testes induced by cadmium toxicity. - *Biol. Trace Elem. Res.,* **153**(1–3), 2013, 269-278.
16. **Gunnarsson, D., G. Nordberg, G., Selstam.** Differential effects of cadmium on the gene expression of seven-transmembrane-spanning receptors and GAPDH in the rat testis. – *Toxicol. Lett.,* **168,** 2007, 51-57.
17. **Hew, K. W., W. A. Ericson, M. J. Welsh.** A single low cadmium dose causes failure of spermiation in the rat. - *Toxicol. Appl. Pharmacol.,* **121,** 1993, 15-21.
18. **IARC**, IARC monographs vol. 100C. - *In: Evaluation of carcinogenic risks to humans,* International Agency for Research on Cancer, Lyon, France, 2012.
19. **Ige, S. F., S. B. Olaleye, R. E. Akhigbe, T. A. Akanbi, O. A. Oyekunle, U. A. Udoh.** Testicular toxicity and sperm quality following cadmium exposure in rats: Ameliorative potentials of Allium cepa. - *J. Hum. Reprod. Sci.,* **5**(1), 2012, 37-42.
20. **Järup, L., M. Berglund, C. G. Elinder, G. Nordberg, M. Vahter.** Health effects of cadmium exposure. A review of the literature and a risk estimate. - *Scand. J. Work Environ. Health,* **24**(1), 1998, 1-51.
21. **Ji, Y. L., H. Wang, P. Liu, X. F. Zhao, Y. Zhang, Q. Wang, H. Zhang, C. Zhang, Z. H. Duan, C. Meng, D. X. Xu.** Effects of maternal cadmium exposure during late pregnant period on testicular steroidogenesis in male offspring. - *Toxicol. Lett.,* **205**(1), 2011, 69-78.
22. **Kaji, T., C. Yamamoto, S. Miyajima, M. Suzuki, Y. Fujiwara, M. Sakamoto, F. Koizumi.** Vascular smooth muscle cells in culture are highly sensitive to cadmium cytotoxicity without species related differences: comparison with Chang liver cells. - *Biol. Pharm. Bull.,* **18,** 1995, 1392-1395.
23. **Kara, H., A. Cevik, V. Konar, A. Dayangac, M. Yilmaz.** Protective effects of antioxidants against cadmium-induced oxidative damage in rat testes. - *Biol. Trace. Elem. Res.,* **120,** 2007, 205-239.
24. **Kishimoto, T., T. Oguri, S. Yamabe, M. Tada.** Effect of cadmium injury on growth and migration of cultured human vascular endothelial cells. - *Hum. Cell,* **9,** 1996, 43-48.
25. **Kishimoto, T., Y. Fukuzawa, M. Abe, M. Isobe, M. Hashimoto, M. Tada.** Cadmium injury of cultured human vascular endothelial cells. - *Hum. Cell,* **4,** 1991, 329-334.
26. **Kolluru, G. K., K. P. Tamilarasan, P. Geetha, N. Durgha, S. Chatterjee.** Cadmium induced endothelial dysfunction: consequence of defective migratory pattern of endothelial cells in association with poor nitric oxide availability under cadmium challenge. - *Cell Biol. Int.,* **30,** 2006, 427-438.

27. **Kubota, H., M. Avarbock, R. Brinster.** Growth factors essential for selfrenewal and expansion of mouse spermatogonial stem cells. - *Proc. Natl. Acad. Sci. U. S. A.,* **101,** 2004, 16489-16494.
28. **Lafuente, A., A. Gonzalez-Carracedo, A. Romero, P. Cano, A. I. Esquifino.** Cadmium exposure differentially modifies the circadian patterns of norepinephrine at the median eminence and plasma LH, FSH and testosterone levels. - *Toxicol. Lett.,* **146,** 2004, 175-182.
29. **Lafuente, A., P. Cano, A. Esquifino.** Are cadmium effects on plasma gonadotropins, prolactin, ACTH, GH and TSH levels, dose-dependent? - *BioMetals,* **16,** 2003, 243-250.
30. **Laskey, J. W., P. V. Phelps.** Effect of cadmium and other metal cations on in vitro Leydig cell testosterone production. - *Toxicol. Appl. Pharmacol.,* **108,** 1991, 296-306.
31. **Li, J. L., R. Gao, S. Li, J. T. Wang, Z. X. Tang, S. W. Xu.** Testicular toxicity induced by dietary cadmium in cocks and ameliorative effect by selenium. - *Biometals,* **23,** 2010, 695-705.
32. **Luca, D., C. Lilli, C. Bellucci, F. Mancuso, M. Calvitti, I. Arato, G. Falabella, S. Giovagnoli, M. C. Aglietti, A. Lumare, G. Muzi, R. Calafiore, M. Bodo.** Toxicity of cadmium on Sertoli cell functional competence: an in vitro study, - *J. Biol. Regul. Homeost. Agents,* **27**(3), 2013, 805-816.
33. **Marettov, E., M. Maretta, J. Legáth.** Toxic effects of cadmium on testis of birds and mammals: a review. - *Animal reproduction science,* **155,** 2015, 1-10.
34. **Marettová, E., M. Maretta, J. Legáth.** Changes in the peritubular tissue of rat testis after cadmium treatment. - *Biol. Trace Elem. Res.,* **134,** 2010, 288-295.
35. **Martin, M. B., H. J. Voeller, E.P. Gelmann, J. Lu, E. G. Stoica, E. J. Hebert, R. Reiter, B. Singh, M. Danielsen, E. Pentecost, A. Stoica.** Role of cadmium in the regulation of AR gene expression and activity. - *Endocrinology,* **143**(1), 2002, 263-275.
36. **Meng, J., R. Holdcraft, J. Shima, M. Giswold, R. Braun.** Androgens regulate the permeability of the blood–testis barrier. - *Proc. Natl. Acad. Sci. U. S. A.,* **102,** 2005, 16696-16700.
37. **Mruk, D. D., C. Y. Cheng.** Environmental contaminants. Is male reproductive health at risk? - *Spermatogenesis,* **1,** 2011, 1-8.
38. **Nair, A. R., O. de Gheselle, K. Smeets, E. van Kerkhove, A. Cuypers.** Cadmium-induced pathologies: where is the oxidative balance lost (or not)? – *Int. J. Mol. Sci.,* **14,** 2013, 6116-6143.
39. **Niewenhuis, R. J., C. Dimitriu, W. C. Prozialeck.** Ultrastructural characterization of the early changes in intercellular junctions in response to cadmium (Cd2+) exposure in LLCPK1 cells. - *Toxicol. Appl. Pharmacol.,* **142,** 1997, 1-12.
40. **Patra, R. C., A. K. Rautray, D. Swarup.** Oxidative stress in lead and cadmium toxicity and its amelioration. - *Vet. Med. Int.,* 2011, http://dx.doi.org/10.4061/2011/457327.
41. **Pavlova, E., J. Ivanova, D. Dimova, Y. Gluhcheva, N. Atanassova.** Alterations in adult mouse testis after subacute intoxication with cadmium and monensin detoxication. - *Eur. Chem. Bull.,* **1**(11), 2012, 463-465.
42. **Prozialeck, W. C., J. Edwards, J. M. Woods.** The vascular endothelium as a target of cadmium toxicity. - *Life Sci.,* **79,** 2006, 1493-1506.
43. **Rensen, S. S. M., P. van Doevendans, G. Eys.** Regulation and characteristics of vascular smooth muscle cell phenotypic diversity. - *Neth. Heart J.,* **15,** 2007, 100-108.
44. **Sarkar, A., G. Ravindran, V. Krishnamurthy.** A brief review on the effect of cadmium toxicity: from cellular to organ level. - *Int. J. Biotech. Res.,* **3,** 2013, 17-36.
45. **Sen Gupta, R., J. Kim, C. Gomes, S. Oh, J. Park, W. B. Im, J. Y. Seong, R. S. Ahn, H. B. Kwon, J. Soh.** Effect of ascorbic acid supplementation on testicular steroidogenesis and germ cell death in cadmium-treatedmale rats. - *Mol. Cell Endocrinol.,* **221,** 2004, 57-66.
46. **Setchell, B. P., G. M. H. Waites.** Changes in the permeability of the testicular capillaries and of the "blood–testis barrier" after injection of cadmium chloride in the rat. – *J. Endocrinol.,* **47,** 1970, 81-86.
47. **Singh, P., S. Chaudhary, A. Patni, V. Sankhla.** Effect of cadmium chloride induced genotoxicity in bone marrow chromosomes of swiss albino mice and subsequent protective effects of Emblica officinalis and vitamin C. - *J. Herb. Med. Toxicol.,* **1**(2), 2007, 67-71.
48. **Siu, E. R. , E. W. Wong, D. D. Mruk, K. L. Sze, C. S. Porto, C. Y. Cheng.** An occludin-focal adhesion kinase protein complex at the blood-testis barrier: a study using the cadmium model. - *Endocrinology,* **150**(7), 2009, 3336–3344.
49. **Siu, E. R., D. D. Mruk, C. S. Porto, C. Y. Cheng.** Cadmium-induced testicular injury. - *Toxicol. appl. pharmacol.,* **238**(3), 2009, 240-249.

50. **Stoica, A., B. S. Katzenellenbogen, M. B. Martin.** Activation of estrogen receptor-alpha by the heavy metal cadmium. - *Mol. Endocrinol.,* **14**(4), 2000, 545-553.
51. **Tam, P., W. Liu.** Gonadal development and fertility of mice treated prenatally with cadmium during the early organogenesis stages. - *Teratology,* **32**(3), 1985, 453-462.
52. **Thompson, J., J. Bannigan.** Cadmium: toxic effects on the reproductive system and the embryo. - *Reprod. Toxicol.,* **25**(3), 2008, 304-315.
53. **Waalkes, M. P., C. D. Klaassen.** Concentration of metallothionein in major organs of rats after administration of various metals. - *Fundam.Appl. Toxicol.,* **5,** 1985, 473-477.
54. **Wang, R., S. Yeh, L. Chen, H. Lin, C. Zhang, J. Ni, C. Wu, P. A. di Sant'Agnese, M. de Mesy-Bentley, C. Tzeng, C. Chang.** Androgen receptor in Sertoli cell is essential for germ cell nursery and junctional complex formation in mouse testes. - *Endocrinology,* **147,** 2006, 5624-5633.
55. **World Health Organization. Regional Office for Europe.** Air quality guidelines. World Health Organization. Regional office for Europe, Copenhagen, Denmark, 2000.
56. **Yang, J. M., M. Arnush, Q. Y. Chen, X. D. Wu, B. Pang, X. Z. Jiang.** Cadmium induced damage to primary cultures of rat Leydig cells. - *Reprod. Toxicol.,* **17,** 2003, 553-560.
57. **Yu, X., S. Hong, E. M. Faustman.** Cadmium-induced activation of stress signaling pathways: disruption of ubiquitin-dependent protein degradation and apoptosis in primary rat Sertoli cell-gonocyte cocultures. - *Toxicol. Sci.,* **104,** 2008, 385-396.
58. **Zhou, T., X. Jia, R. E. Chapin, R. R. Maronpot, M. W. Harris, J. Liu, M. P. Waalkes, E. M. Eddy.** Cadmium at a nontoxic dose alters gene expression in mouse testes. - *Toxicol. Lett.,* **154,** 2004, 191-200.