Cadmium Exposure and Testis Susceptibility: a Systematic Review in Murine Models

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Abstract

It is known that cadmium induces damage to the testis. However, the significant cadmium impact on the testicular architecture and the mechanisms involved in this process are not clear. Besides, the relationship between dose, route, and time of exposure and injuries remains poorly understood. Thus, we aimed to assess whether cadmium exposure in any dose, route, and time of exposure causes significant alteration in the testicular tissue of murine models, as well as the main mechanisms involved. We performed a structured search on the Medline/PubMed and Scopus databases to retrieve studies published until September 2018. The results were organized into an Adverse Outcome Pathway (AOP) framework. Also, a bias analysis of included studies was performed. We included 37 studies, and most of them identified significant histopathologies in both tubule and intertubule regarding routes, in a dose- and time-dependent manner. The damages were observed after the first hours of exposure, mainly vascular damages suggesting that vasculature failure is the primary mechanism. The AOP showed that potential molecular initiating events may mimic and interfere with essential elements disrupting proteins (structural and antioxidants), change in the oxidative phosphorylation enzyme activities, and gene expression alteration, which lead to reproductive failure (adverse outcome). Analysis of methodological quality showed that the current evidence is at high risk of bias. Despite the high risk of bias, cadmium triggers significant lesions in the testis of murine models, regarding routes, in a dose- and time-dependent manner, mainly due to vascular changes. Therefore, cadmium is a risk factor for male reproductive health.

Keywords Reproductive toxicology · Environmental contaminant · Adverse outcome pathway (AOP) · Vascular damage · Male reproductive health

Introduction

Cadmium (Cd) is of great concern due to its ubiquitous and non-biodegradable characteristics, toxicity, and tendency of bioaccumulation [1, 2]. Human beings are daily exposed to this ubiquitous environmental toxicant through non-occupational sources, including food and water ingestion, and occupational sources, mainly in processes involving heating cadmium-containing materials such as the production of alloys and batteries [3, 4]. Cadmium has been reported to cause several health disorders [5, 6], and at the same time, male infertility has been described as a global and significant health problem [7]. Among the causes of male infertility, some substances have shown negative effects on spermatogenesis and sperm quality [8]. In this context, Cd is one of the most investigated heavy metals that play a key role in male infertility due to the association between its high concentration in seminal plasma and decreased sperm quality in infertile men [9, 10].

Due to the exceptionally long half-life, 10 to 35 years, Cd accumulates in the body and can cause injuries [11, 12]. The Cd-induced reproductive toxicity has been reported, even at

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low doses and brief exposure [13, 14]. Testes are exceedingly susceptible to this persistent heavy metal, and the harmful effect is usually related to multiple mechanisms, such as inflammation, cytotoxicity, oxidative stress, interference with selected signaling pathways, epigenetic regulation of genes, and disruption of the hypothalamus-pituitary-gonadal axis. The testicular injuries include structural damage to the seminiferous epithelium, germinative and somatic cells, blood-testis barrier, and testis vasculature [15, 16]. In this context, understanding the histopathological changes of test is after Cd exposure and the molecular and biochemical mechanisms involved in these alterations provide useful information about this toxic agent's impact on male reproductive health [17].

Currently, it is known that Cd induces testicular damages [18]. However, the significant impact of Cd on the testicular architecture and the mechanisms involved in this damaging process are not clear. Besides, it remains poorly understood if there is a relationship between dose, route, and time of exposure and the injury intensity. Thus, this information is extremely important to provide a direction for future researches in this field and the development of decision making for therapeutic alternatives on the treatment of testicular injuries. Thereby, based on the published data associating Cd and reproductive toxicology and our knowledge that the testis is susceptible to heavy metal-induced toxicity, we hypothesize that Cd can promote significant injuries in the testicular tissue by several routes, doses, and time of exposure in murine models.

Therefore, considering the fragmented knowledge, we performed this systematic review to assess whether Cd exposure (in any dose, route, and time of exposure) causes significant testicular tissue alterations, including any outcome of testicular histomorphology, as well as molecular, biochemical, and hormonal evaluations performed in order to understand the mechanisms involved in the histomorphological changes, in murine models. We also aimed at reporting what types of histomorphological analyses have been used to understand these effects. Given the uncertainties and controversies surrounding the action of Cd exposure in the testicular damage, the results of this review were organized into an adverse outcome pathway (AOP) framework to provide a systematic and transparent assembly of the evidence. In addition, the AOP's framework constructed in this review allowed the identification of the potential molecular initiating events (MIE) involved in the testicular damage process and the consequences to male reproduction health. The results of this study may help to understand the main mechanisms involved in the alterations triggered by other heavy metals, even other environmental contaminants, and, consequently, the translation to the human health risk assessment. Based on a detailed analysis of methodological bias, we also evaluated the force of the evidence by analyzing the advances and limitations of the studies carried out in this field.

Materials and Methods

This systematic review was conducted based on PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) [19]. Details of the protocol for this systematic review were registered on PROSPERO—International prospective register of systematic reviews (CRD42019158315). Details of the Population, Exposure, Comparators, and Outcome (PECO) are given in Online Resource 1—Table S1.

Focus Question

What are the significant effects of Cd exposure considering several doses, routes, and time of exposure in the testicular tissue of adult murine models? What are the main testicular tissue alterations including any outcome of the testicular histomorphology and consequently, other molecular, biochemistry, and hormonal evaluations involved in these processes?

Search Strategy

An extensive bibliography search was performed using the electronic databases Medline/PubMed (https://www.ncbi.nlm.nih.gov/pubmed) and Scopus (https://www.scopus.com/home.uri), completed on September 21, 2018, at 2:13 p.m. For all databases, the search filters were based on three complementary levels: (i) animals, (ii) testis, and (iii) cadmium, which were combined by Boolean connectors [AND] (Online Resource 1—Table S2). Search filters were initially developed for PubMed. The search algorisms [MeSH Terms] and [TIAB] were applied to identify indexed records and those recently published in an indexing process, respectively. To detect all *in vivo* animal model studies in PubMed, a standardized and optimized animal filter was used [20]. The terms used to search on PubMed were adapted to Scopus for recovery of studies, and the "animals" filter was provided by the website itself.

As search strategies within both databases, the keywords "English" and "male" were selected because it is the official scientific language and to increase the search specificity, respectively. In addition, a backward search (hand-search) was performed, in which the reference list of each included study was manually screened for additional eligible studies that were not retrieved by our search.

Selection Criteria

After record identification through both databases, the duplicate studies were removed. Then, an initial selection based on title and abstract was performed. In this initial selection, we included pre-clinical studies in murine models that assessed

the Cd effect on testicular architecture that did or did not perform molecular, biochemical, and/or hormonal analyses. All timings, frequencies, routes, and dosages of Cd (and compounds) exposure were eligible for inclusion. We excluded studies that did not evaluate the Cd exposure in the testicular histomorphology of murine models. Secondary (literature reviews, letters to the editor, case studies, comments, and editorials) and *in vitro* studies were also excluded. After the initial screening, all relevant studies were recovered in full text and evaluated by the eligibility criteria. We excluded studies that either had no available full text was not available or did not meet the criteria described above.

Evidence Synthesis

Considering our objectives, data extraction was based on descriptive levels as follows: (i) characteristics of publication: authors, publication year, and country; (ii) characteristics of the experimental animals: animal model, age, weight, number of animals, number of animals per group, and number of groups; (iii) exposure: compounds, doses, periodicity of administration, route, duration, and existence of a control group; (iv) main histomorphological outcomes and analyses as well as the main molecular, biochemical, and hormonal results related with the histomorphological alterations; and (v) secondary outcomes. We classified the main histomorphological analyses in two levels: (i) qualitative analyses, in which the study reports the Cd effect in a descriptive way using words such as presence/absence, yes/no, and (ii) quantitative analyses, in which the study reports the Cd effect in an objective way related with numbers that are absolute value/percentage usually being the mean of the group. After the extraction of outcomes, which were identified as "key events," an AOP analysis was performed considering the main outcomes reported by included studies as well as how these key events are related to each other and how they can interact [21].

Bias Analysis

The quality of the studies was assessed by the criteria described on the SYRCLE's Risk of Bias (RoB) tool (Systematic Review Centre for Laboratory Animal Experimentation) designed specifically for animal studies [22]. The following methodological domains based on RoB were evaluated considering the following: selection bias—"Was the allocation sequence adequately generated and applied?", "Were the groups similar at baseline or were they adjusted for confounders in the analysis?", "Was the allocation to the different groups adequately concealed?"; performance bias—"Were the animals randomly housed during the experiment?", "Were the caregivers and/or researchers blinded regarding which intervention each animal received during the experiment?"; detection bias—"Were animals

selected at random for outcome assessment?", "Was the outcome assessor blinded?"; attrition bias—"Were incomplete outcome data adequately addressed?"; reporting bias—"Are reports of the study free of selective outcome reporting?"; other biases—"Was the study apparently free of other problems that could result in high risk of bias?". The items in the RoB tool were scored with "yes" (low risk of bias); "no" (high risk of bias); or "unclear" (indicating that the item was not adequately reported, and therefore, the risk of bias was unknown). Based on these items, we constructed a figure in the Review Manager 5.3 program, based on Cochrane Collaboration (RoB 2.0), to demonstrate the risk of bias across all studies included.

## Results

Included Studies

Our search strategy allowed recovering 2112 records (MEDLINE—1277 and Scopus—835). After the removal of 653 duplicates, 1459 records were screened by reading the title and abstract. Then, 1410 studies were excluded due to inappropriate topics. Forty-nine studies were assessed through the eligibility criteria, of which 30 were included. The reference list screening resulted in 7 other records that met the eligibility criteria. Therefore, 37 records [23–59] were included in this systematic review (Fig. 1).

Characteristics of Publication, Experimental Animals, and Cd Exposure

The studies were published between 1959 and 2017, and they were conducted in several countries, including USA (24.32%), Brazil (10.81%), India, and Spain (8.11% each). Considering animal models, rats were the most used (64.87%), followed by mice (29.73%) and wild rodents (5.41%). Among the studies with rats, Wistar rats were the animal strain most used (54.17%). Among mice, Swiss mice were prevalent (54.55%), and two gerbil species were used in the wild rodents (Online Resource 1—Table S3).

Concerning the metal exposure, cadmium chloride ($CdCl_2$) was the widely used compound (91.89%). The lowest compound doses used were 0.00005 mg/kg BW (body weight) $CdCl_2$ (0.00003 mg/kg Cd) (oral (*Or*), daily dose for 6 months) [37], and 5 μM/kg BW $CdCl_2$ (0.56 mg/L Cd) (intraperitoneal (*Ip*), single dose) [43]. The highest compound doses used were 326.2 mg/kg BW $CdCl_2$ (200 mg/kg Cd) (*Or*, single dose) [29] and 1121.9 mg/L $CdCl_2$ (688 mg/L Cd) (*Or*, *ad libitum* for 70–80 days) [55]. Most studies assessed the Cd effect administrated in a single dose (54.05%), followed by studies that provided Cd *ad libitum* in drink water or food (18.92%). The subcutaneous route

Fig. 1 Flow diagram of the results of the systematic review literature search. Based on PRISMA statement "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (www.prisma-statement.org)
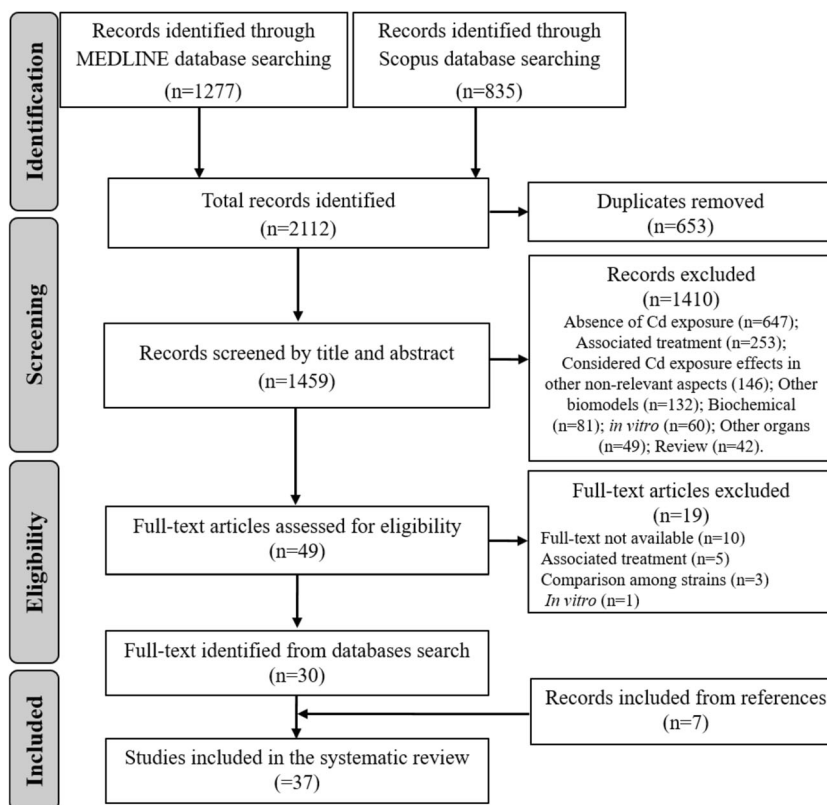
(Sc) was the most used route of administration (32.43%), followed by *Ip* and *Or* (24.32% each). The experiments lasted from 2 h to 30 months. About the control group, almost 22% of the records did not specify if the animals received something as placebo, and in 8.11%, the control group did not receive any treatment (Online Resource 1—Table S4).

Main Alterations in the Testicular Tissue (Histopathological Outcomes)

The Cd effects on testicular histomorphology are shown in Fig. 2. Almost 83% of the studies that evaluated both interstitial tissue (intertubule) and seminiferous tubules identified damage in both compartments. Approximately 19% of studies evaluated exclusively the seminiferous tubules and all verified Cd-induced damage in this compartment, and 5% evaluated solely the interstitial tissue and all found injuries. Almost 67% of the studies that evaluated the tunica albuginea found injuries such as thickening and calcification. Only one included study did not observe any testicular histological alteration after Cd exposure.

Interstitial Tissue

A total of 31 studies evaluated the interstitial tissue, and almost 87% found damage in this compartment. The main interstitial injuries seen by the studies were hemorrhage, edema,

fibrosis, disorganization (collagen fibers), necrosis, and inflammation. In addition to qualitative outcomes, changes in the percentage of this tissue were confirmed by quantitative analyses performed by studies that used this method.

Among the 23 studies that evaluated Leydig cells, 65% reported alterations in these interstitial cells such as degeneration and the presence of tumors (generally considered adenomas). In addition, death in the Leydig cells was also reported in which necrosis was confirmed. The quantitative analyses showed a decrease in nuclei, cytoplasm, volume, and Leydig cell percentage.

Blood vessels were also investigated by 18 studies, and almost 83% confirmed that they are also targets for Cd-induced toxicity. Hyperemia was the most prevalent disorder, followed by thrombosis. Besides, dilatation of blood vessels was also verified by some studies. Ultrastructural changes in the blood vessels, such as tumefaction of endothelial cells, increase of pinocytotic vesicles, and loss of integrity of the desmosome complex were also reported. The quantitative analysis confirmed increase of lumen vessels.

Seminiferous Tubules

Similarly to that observed in the interstitial tissue, it was reported that Cd caused several alterations to the seminiferous tubules. A total of 35 studies evaluated this compartment, and almost 91% found damages. The main injuries reported were

Fig. 2 Main outcomes of Cd exposure on testicular histomorphology in murine models. Vol. = volume; GCs = germ cells

necrosis, calcification/mineralization, atrophy, and the presence of multinucleated giant cells. Degeneration of the seminiferous tubules was also observed. Some studies also verified that the damaged tubular areas were replaced by another tissue (fibrous connective tissue or amorphous mass). The quantitative analyses reported changes in the length, diameter, volume, and percentage of seminiferous tubules, and increase of impaired tubule number.

The germinal epithelium was greatly impaired by Cd administration, mainly the germ cells. Almost a third of the studies that evaluated the seminiferous tubules verified Cd-induced death in this cellular type. Other damages were also observed at the cellular level in the germ cells such as vacuolization and heterochromatic and disordered nuclei. The injuries possibly led to the decrease of these cells and their layers, which was also reported. After Cd exposure, the germinal epithelium was reported disorganized, with detachment and/or loss, and vacuolization. The Sertoli cells were also reported as a target of Cd-induced toxicity. Damage at the cellular level, such as vacuolization and disruption of blood-testis barrier, and Sertoli cell death were confirmed. The decrease of the germinal epithelium and spermatogenic index and the increased failure of spermiation and detachment were also confirmed by quantitative analyses.

Considering the lumen changes observed by studies that analyzed the seminiferous tubules, they are described as spermatozoa reduction, lumen obliteration, and a significant increase in its percentage. In addition to seminiferous tubule injuries, some records observed that the tunica propria was disintegrated and thickened.

Main Histomorphological Analyses

Most studies (70.27%) assessed the Cd effects on testicular tissue by qualitative analyses (i.e., descriptive analysis related to words—presence/absence, yes/no). However, quantitative analyses (i.e., objective analysis related to numbers—absolute value/percentage) were also used (13.51%) and some studies (16.22%) used both methodologies.

Regarding seminiferous tubules, approximately 90% of the studies that evaluated this compartment by qualitative analyses reported alterations, and almost 78% of the studies that used quantitative analyses found some tubular changes. Concerning interstitial tissue, 86% of the studies that evaluated this tissue by qualitative methods verified some injuries, and all studies that used quantitative analyses found some alterations in this tissue (Fig. 2).

Correlation Between Study Characteristics and Testicular Tissue Alterations

The characteristics of the studies, mainly the experimental animal model, route, dose, and time of exposure, play a crucial role in the Cd-induced testicular toxicity. Considering the animal model, all studies that used rats looked for seminiferous tubular alteration, in which almost 92% found some injuries in this compartment, and 20 studies looked for intertubular alteration in which 85% found injuries. About mice, among the studies that used this animal model, 81.82% observed the seminiferous tubules and all reported injuries, and 9 studies looked for alteration in the interstitial tissue, which was confirmed by 88.89%. It is important to point out that several alterations observed in both seminiferous tubules and interstitial tissue (Fig. 2) were not specific to any animal model.

Regarding doses, specifically, the lowest Cd compound doses in which histomorphological alteration was reported were 0.005 mg/kg BW CdCl$_2$ (0.003 mg/kg Cd) (*Or*, daily for 6 months) [37] and 15 μM/kg BW CdCl$_2$ (1.68 mg/L Cd) (*Ip*, single dose, 48 h) [43]. Damages at germ cells (pyknosis) and detachment of the germinal epithelium were reported by these studies, respectively. In addition, 45.95% of the studies compared low and high dose-response effects and most of them confirmed that the histopathological outcomes were dose-dependent such as epithelial damages, mainly death of germ cells, and interstitial damages, for example, Leydig cell tumors. Furthermore, when some studies compared several dose frequencies, they observed that a high single dose administration, at the same amount of Cd or even less, is worse than several low doses. They concluded it by the increase of calcification and necrosis of the seminiferous tubules observed by high single Cd doses.

Regarding routes, all studies that used the *Sc* route evaluated the seminiferous tubules and approximately 92% observed some alterations, and ten records analyzed the interstitial tissue, which was reported with changes by 90% of them. All the studies that used the *Ip* route evaluated the seminiferous tubules and found some alterations, while eight of them evaluated the interstitial tissue and damage was confirmed by almost 88%. Six studies that used the *Or* route observed the seminiferous tubules, and damages were reported by 67% of them. Damages in the interstitial tissue were reported by 75% of studies that evaluated this compartment and used the *Or* route.

Regarding the exposure time, although there is not a consensus between chronic and acute exposures, we considered acute exposure until 4 weeks and after this chronic exposure. Fourteen studies evaluated acute Cd exposure, in which all found injuries, and 13 studies evaluated chronic Cd exposure, in which 92% found changes. In addition, ten studies assessed both acute and chronic Cd effects, in which only 20% of them did not report alteration after both exposures.

In acute exposure, seven records evaluated Cd-induced toxicity in the first 2 to 24 h and almost 71% found early morphological changes mainly in the blood vessels, such as loss of integrity of the desmosome complexes (after 2 h) and thrombosis (after 14 h). Edema and hemorrhage (after 4 h) were also seen in the interstitial tissue. Fifteen studies observed the testis between 2 and 7 days of Cd exposure. The injuries were similar to those described initially, with some of them being in a time-dependent manner, as the failure of spermiation. In addition, at this stage, the studies also reported more tubular alterations, such as necrosis, calcification, and even damage to Sertoli cells. Lyses and decrease of Leydig cells were also confirmed. All of 12 studies that evaluated the testis between 10 days and 4 weeks of Cd exposure also reported damages. Interstitial injuries such as hemorrhage and necrosis were observed, as well as tubular damage such as degeneration of germinal epithelium, germ cell necroses, and damage at Sertoli cells.

Lastly, almost 92% of the studies that assessed the chronic Cd effects (between 35 days and 33 months) reported several histopathological changes in both testicular compartments. Extensive tubular necroses, calcification, decrease of germ cells, and vacuolization of Sertoli cells were the main tubular alterations, while in the interstitial tissue hemorrhage, fibrosis, and damage in the Leydig cells as tumors were confirmed.

Main Mechanisms Involved in the Histopathologies: an AOP Framework

Some records performed molecular, chemical, and/or hormonal analyses to answer if the Cd-induced toxicity affects other parameters in order to understand the histomorphological alterations. Approximately 16% of the studies [31, 32, 44, 48, 52, 56] evaluated the testis at the molecular level and observed some modifications, such as increase of DNA oxidation with increase of apoptotic index [31] and increase of expression of autophagy-related proteins (e.g., *Beclin 1*) that are associated with testicular injuries [52]. In addition, another study reported increased expression of *C-myc* and *Egr1* genes suggesting stress response, and repressed expression of pro-apoptotic (e.g., *Casp3*) and DNA repair genes (e.g., *Msh2*), which possibly also contributes to Cd-induced carcinogenesis [56].

The oxidative stress markers were investigated [30, 38], which detected increased antioxidant enzyme activities, such as catalase, superoxide dismutase, and/or total glutathione. This increase suggests that the upregulation of antioxidant enzymes was due to increased generation of reactive oxygen species (ROS), which could justify some alterations such as in the vascular tonus. An increase of lipid and protein peroxidation markers was also observed by these studies, which also contributes to injuries in the tissue. One study assessed the essential mineral content in which microminerals

such as selenium (Se), copper (Cu), Iron (Fe), and magnesium (Mg) were significantly reduced, while the calcium (Ca) concentration increased, which justified the calcification seen by this study [30

In addition, changes in the adiponectin levels were verified, which can affect the testosterone levels or can be affected by it [31]. A decrease of vimentin in fibroblasts and endothelial cells was also reported [40]. Change in the activity of some oxidative phosphorylation enzymes (triphosphatase and succinic dehydrogenase) in seminiferous tubules was also confirmed and can lead to increase of ROS [50]. Regarding the hormonal evaluation, the results showed disturbances in luteinizing hormone (LH), follicular stimulating hormone (FSH), and/or testosterone levels, observing that the downregulation of steroidogenesis can affect or be affected by morphological changes, mainly in Leydig cells [31, 39, 57].

The main histomorphological outcomes as well as the molecular, chemical, and hormonal results were organized in an AOP network to provide a systematic and transparent assembly of the evidence regarding Cd effects in the testicular tissue. It was possible to identify by molecular and chemical outcomes three potential molecular initiating events (MIE): (i) mimicry and interference of Cd with essential elements, which disrupt some essential element-dependent proteins (structural or antioxidant enzymes); (ii) change in the activity of oxidative phosphorylation enzymes; and (iii) gene expression alteration. The first cited MIE can disrupt directly the endothelial cells, causing loss of junctional complex integrity (e.g., desmosomes), in which the first histopathologies are related to circulatory failure. These MIE trigger mainly increase in ROS levels and, consequently, oxidative stress, which leads to several histomorphological alterations and even death in endothelial, Leydig, germ, and Sertoli cells. Changes in the testis weight and appearance proceed these alterations as observed by some included studies. All these key events lead to a significant decrease of spermatogenesis and steroidogenesis resulting in reproductive failure (Fig. 3).

Blood and Testicular Cadmium Concentrations

In a complementary manner, 15 studies verified the Cd concentration in the testis and/or blood after exposure. Almost 87% of them measured the amount of Cd in the testis and 20% in blood. Of these that measured the Cd amount, only one did not detect Cd in the testis. However, 60% of the studies did not measure the tissue concentration of Cd after exposure(Fig. 4).

Risk of Bias

Figure 5 shows the percentage of each risk of bias item across all included studies. The result for the risk of bias assessment of individual studies is available in Online Resource 1—Fig. S1. No studies fulfilled all methodological criteria analyzed. Ten

studies described the baseline characteristics (strain, weight, and age) among the animals, but most studies ($n = 27$) have some baseline characteristics missing or did not mention whether the experimental groups were similar at baseline characteristics. Twenty-three studies did not mention allocation concealment, and 12 studies reported the randomization of animals among the groups but did not describe the used methods. Two studies did not have more than one experimental group, which presented low risk of bias for this item. More than half of the studies reported that the animals were exposed to standardized room conditions and specified these conditions, whereas in the other studies this information is unclear or not provided. None of the studies reported about random sequence generation, blinding of participants and personnel (caregivers and investigators), random outcome assessment, and blinding of outcome assessment (outcome assessor). Regarding incomplete outcome data, almost 32% of the studies reported whether there was animal exclusion and the reason for the measure, while in the other records this information is not clear, or they did not mention anything about it. Most studies were free of selective outcome reporting. However, some studies did not mention all protocols or did not include all expected outcomes (compared methods and results), which poses a high risk of bias or omitted some important information, which made it unclear. More than half of the studies were seemingly free from other problems that could increase the risk of bias. Conversely, some studies presented unclear risk because either the control animals received nothing as placebo or they did not mention about it. Moreover, some studies presented high risk due to the absence of control or different periods of intervention between control and treatment groups, or they did not report whether wild animals were pathogen-free.

Discussion

In our study, a systematic review was conducted to assess the significant Cd impact on the testicular tissue of adult murine models, in any dose, route, and time of exposure, and the main mechanisms involved in the damage process. Our results revealed strong evidence that Cd exposure induces severe histopathologies in both seminiferous tubules and interstitial tissue of murine models regarding routes, in a dose- and time-dependent manner. The main mechanisms involved in these processes may be related to three potential molecular initiating events (MIE) identified by the adverse outcome pathway (AOP) framework: mimicry and interference of Cd with essential elements, which disrupt some essential element-dependent proteins (structural or antioxidant enzymes); change in the activity of oxidative phosphorylation enzymes; and gene expression alteration. The first reported MIE disrupts directly the endothelial structural proteins, in which the primary mechanism involved in the lesion may be vascular damage. All the MIE contribute to the increase of free radicals and
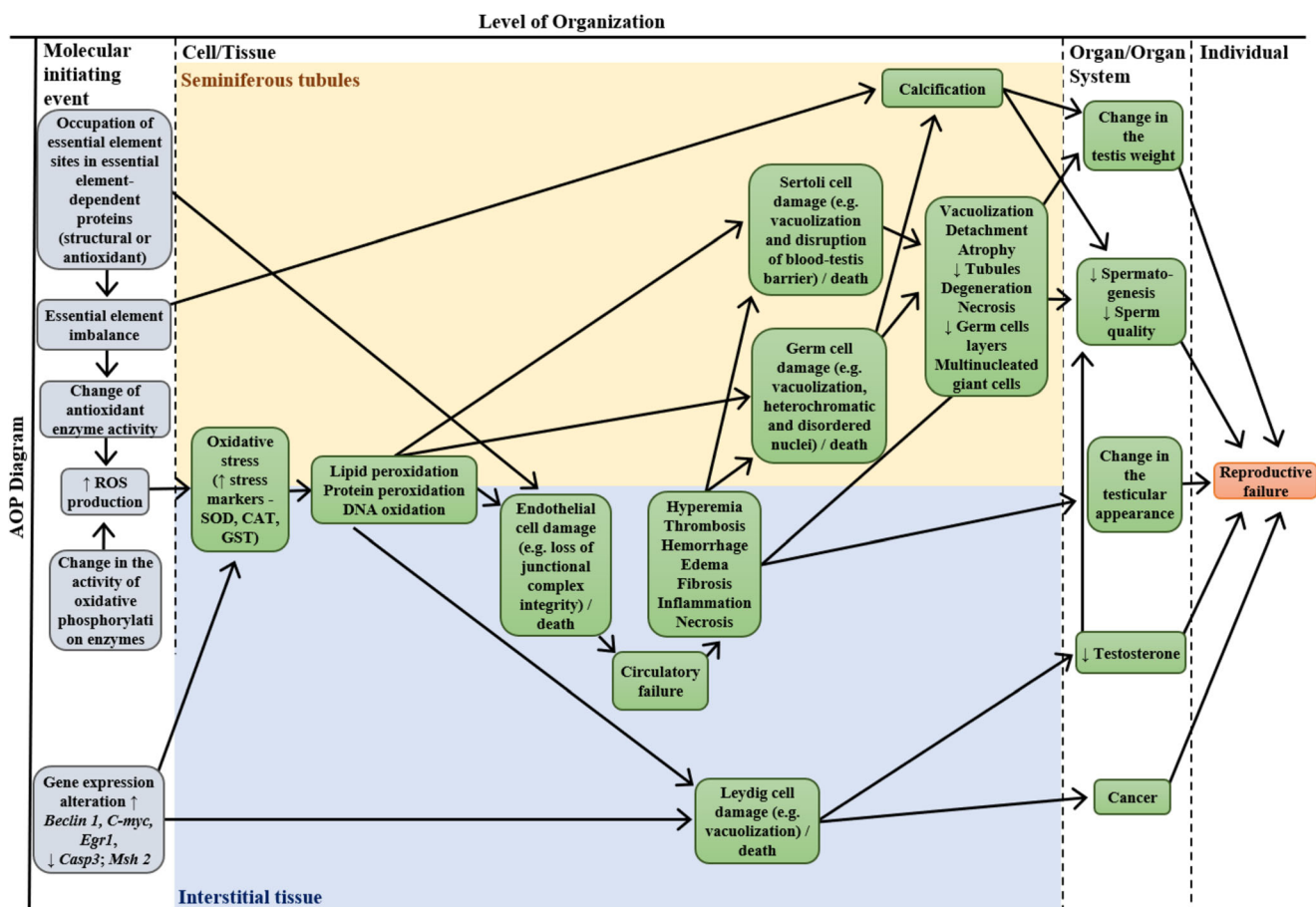
Level of Organization

Fig. 3 Adverse outcome pathway (AOP) analysis based on the main and prevalent results of the included studies. Cadmium (Cd)-induced testicular histopathologies are associated with three potential molecular initiating events (MIE): (i) mimicry and interference of Cd with essential elements, which disrupt some essential element-dependent proteins (structural or antioxidant enzymes); (ii) change in the activity of oxidative phosphorylation enzymes; and (iii) gene expression alteration. The first cited MIE can disrupt directly the endothelial cells, in which the first histopathologies are related to circulatory failure. These MIE trigger mainly increase in ROS levels and, consequently, oxidative stress, which leads to several histomorphological alterations and even death in endothelial, Leydig, germ, and Sertoli cells. Also, the essential element disbalance is directly related to calcification, so this severe histopathology is also observed in acute exposure. All these key events lead to a significant decrease of spermatogenesis and steroidogenesis resulting in reproductive failure. Gray boxes = molecular initiating event; green boxes = key events; orange box = adverse outcome; arrows = key event relationship. ↑ inside the box = increase; ↓ inside the box = decrease; ROS = reactive oxygen species; SOD = superoxide dismutase; CAT = catalase; GST= total glutathione

consequently increase of reactive oxygen species (ROS) production, which leads to oxidative stress in the tissue with alterations in the antioxidant enzyme activities and, therefore, several injuries in the testis. Furthermore, the testis proved to be an accumulation target of this environmental toxicant, which contributes to the development of testicular damages.

Our results showed that the concern about Cd-induced toxicity on testicular histomorphology of murine models has been reported in the literature since 1959 [42]. For this purpose, a wide variety of strains were used by the included records, in which rats were the most commonly used followed by mice. Rats have become a species of choice in toxicological research because of its size and relatively docile and physiological similarities, and mice

are generally more economical [60]. However, it has already been reported that sensitive and resistant murine models may exist in toxicological bioassays [61]. Our findings indicate that there is not a species-sensitivity distribution between rat and mouse in Cd-induced histopathologies, in which the Cd is able to induce injuries regarding the murine model and these histopathologies were not species-specific (Fig. 6 (a)). It is probably because rats tend to be more sensitive for determining toxicological outcomes [61] and mice are more susceptible to stress-induced testicular changes [62], so injuries can be found in both species. This result that is not strain-specific is supported by our AOP analysis, in which Cd can trigger the MIE regarding the murine model leading to
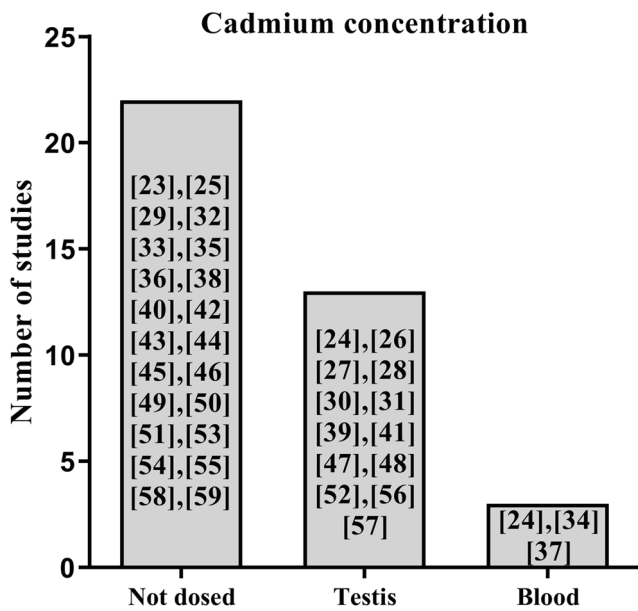
Cadmium concentration

Fig. 4 Studies that did or did not dose the blood and/or testicular cadmium concentration

histopathologies. The sensitivity to Cd-induced testicular toxicity may be related to Cd accumulation [63, 64

The Cd effect on tissue is directly related to doses of exposure, in which in some pathologies, its action appears to be bidirectional and determined by Cd concentration. For example, in tumor angiogenesis, Cd shows to be either stimulatory or inhibitory depending on the concentration [65]. However, our results show that there is strong evidence about testicular histopathological manifestations that are dose-dependent and the damages are intensified following the increase in Cd

concentration. In addition, a high single dose is worse than several low doses, even at the same amount of Cd or less (Fig. 6 (c)).

Regarding routes of exposure, the most common route of environmental Cd exposure in animals and humans is oral (*Or*), and in toxicological studies it is the intraperitoneal (*Ip*) route [66]. However, our findings indicate that the subcutaneous (*Sc*) route is mostly used to evaluate histomorphological alterations, followed by *Ip* and *Or* routes. We believe that the *Sc* route was preferred because it is less aggressive than *Ip*, and it is possible to know the amount of Cd available on the body, differently from the *Or* route in which Cd is absorbed by the duodenum and the amount of Cd that reaches the organism is unknown [67]. Also, our findings indicate that there is strong evidence that Cd exposure can cause testicular histopathologies by all these routes in both tubule and intertubule compartments, even though damages in the seminiferous tubules were more frequently observed by studies that chose direct routes (i.e., *Sc* and *Ip*) other than indirect routes (i.e., *Or*) (Fig. 6 (d)).

Recently, it has already been reported that Cd could affect male reproductive health during both acute and chronic exposure [68], and the severity of Cd-induced reproductive toxicity is also time-dependent [65]. So, our results indicate robust evidence that the Cd-induced testicular toxicity is fast, showing severe testicular damages during the first 24 h or 7 days of exposure. Furthermore, modifications that were expected only in chronic phases such as degeneration, necrosis, and calcification were also observed in the acute phase in the first days of exposure, which support our findings. Some histopathologies were intensified in a time-dependent manner, sustaining that the mechanisms of histopathological injuries start in the acute phase and are increased and enhanced during the chronic phase (Fig. 6 (e)).

Fig. 5 Results of the risk of bias and methodological quality indicators for all included studies that evaluated the Cd impact on testicular histomorphology of murine models. The items of SYRCLE's RoB tool were scored with "yes" (low risk of bias); "no" (high risk of bias); or "unclear" (indicating that the item was not adequately reported, and therefore, the risk of bias was unknown)
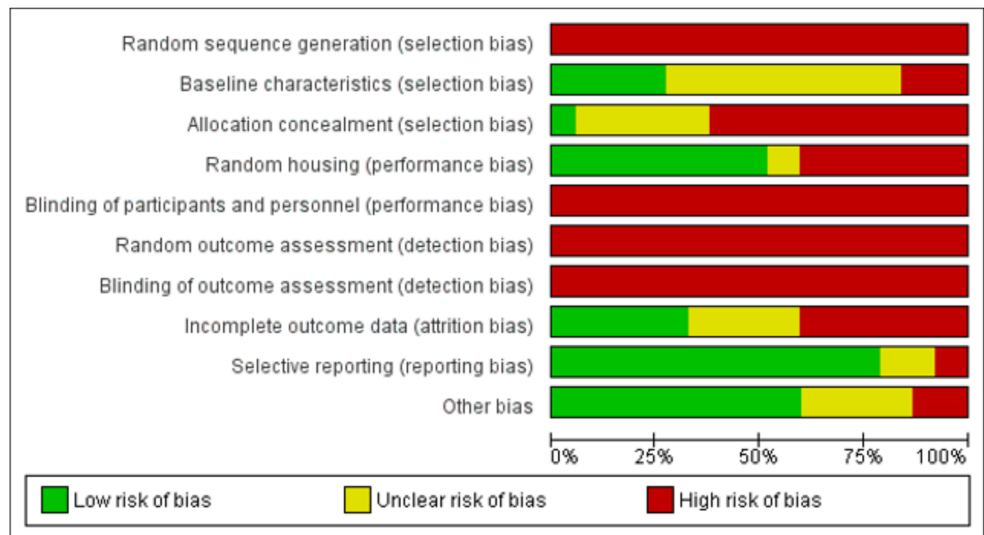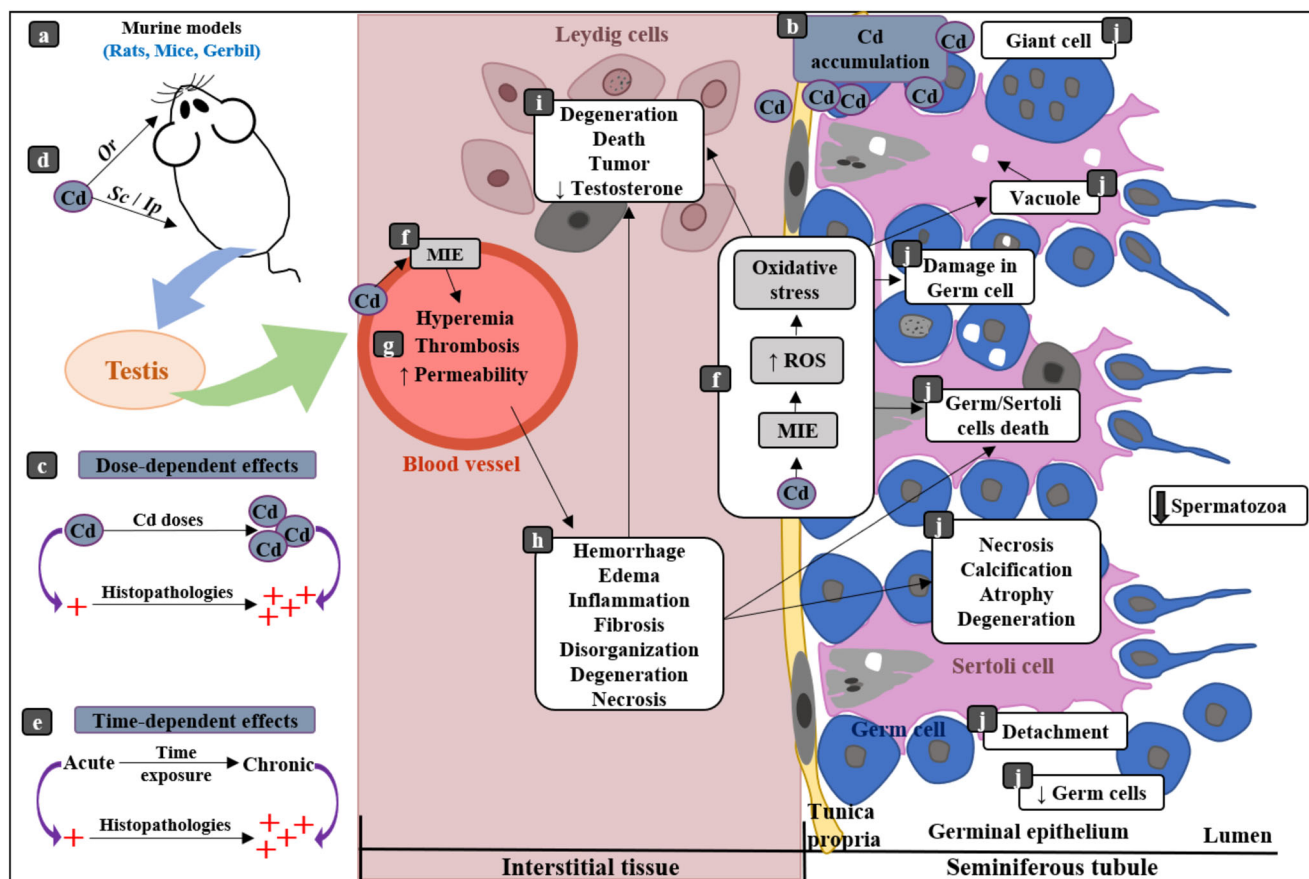
Fig. 6 Current understanding of the Cd effects on the testicular tissue of murine models. (a) All murine models are susceptible to Cd-induced testicular toxicity. (b) Cd accumulation in the testis. (c) The effects of Cd on testicular tissue are dose-dependent. (d) Main routes of Cd administration: *Or* = oral; *Sc* = subcutaneous; *Ip* = intraperitoneal. (e) The effects of Cd on testicular tissue are time-dependent. (f) Molecular initiating events (MIE) identified by the adverse outcome pathway (AOP) framework. (g) Histopathologies related to circulatory failure. (h) Main histopathological secondary consequences of vascular damages in the interstitial tissue. (i) Main histopathological damages in the Leydig cells. (j) Main histopathological damages in the seminiferous tubules including the germ and Sertoli cells. ↑ inside boxes = increase; ↓ inside boxes = decrease

The AOP has been a multifaceted framework that supports the twenty-first-century toxicology [69]. AOP is conceptually synonymous of mode of action developed to analyze the relevance of toxicological effects of chemicals and even non-chemical substances observed in animals to human health risk assessment [21]. In addition, AOP has received substantial attention as an organizing framework for reproductive toxicity [70]. Based on this, we constructed an AOP network to better explain the histopathologies and the main mechanisms involved in these damage processes. So, we scored three potential MIE that may be responsible for causing the main testicular histopathologies that lead to reproductive failure: mimicry and interference of Cd with essential elements, which disrupt some essential element-dependent proteins (structural or antioxidant enzymes); change in the activity of oxidative phosphorylation enzymes; and gene expression alteration (Fig. 6 (f)). Although the evidence is slightly considerable due to few studies that performed molecular and chemical analyses and the high level of bias presented by some of them, this conclusion is in accordance and consistent with

other studies. These MIE have already been reported as the cause of pathologies caused by Cd in other organs [71, 72, 73]

Jointly, it has already been reported that heavy metals can affect on the testes disrupting spermatogenesis and steroidogenesis via mechanisms that involve the increase in ROS production [75, 76]. Cadmium is able to occupy the sites of microminerals, such as zinc (Zn), copper (Cu), and selenium (Se), in the antioxidant enzymes, disrupting their activities and decreasing the micromineral concentration [77, 78]

Our findings indicate that the primary mechanism of Cd-induced disorders on testicular architecture is related to circulatory failure due to endothelial injuries, even in low (single dose of 7.5 mg/kg BW $CdCl_2$ [25]) or high (single dose of 20.25 mg/kg BW $CdCl_2$ [42]) doses, independently of exposure route and animal model. This conclusion is supported by the appearance of interstitial injuries related to vasculature changes after brief Cd exposure (3 h [25]; 12 h [50]; 1 day [49]; 5 days [40]; 7 days [31]), such as hemorrhage, edema, thrombosis, and hyperemia (Fig. 6 (g)). In hyperemia, inflammatory mediators are released

and cause dilatation and increase in the permeability of vessels which leads to the development of an inflammatory process, edema, hemorrhage, ischemia, and, consequently, degeneration and necrosis in the testis [15, 75] (Fig. 6 (h)). Cadmium affects vessel homeostasis by causing structural [25, 40], metabolic, and functional [16, 26, 80] damages at endothelial cells. Since the endothelial junctional complexes are composed of calcium (Ca^{+2})-dependent proteins (e.g., cadherins), the first cited MIE disrupts directly the integrity of junctional complexes (e.g., desmosomes and adherens junctions) [25, 40, 80]. Then, the primary mechanism involved in Cd-induced toxicity may be vascular damage (Fig. 6 (f)). Interestingly, injuries related to circulatory failure are not restricted to short time exposure, indicating that these injuries do not repair if the exposure continues [26, 47]. Therefore, there is strong evidence that blood vessel alterations are the first manifestation of the histopathologies triggered by Cd exposure, in which this mechanism is activated in the acute phase and continued on the chronic phase.

In addition to injuries in the blood vessels, the main histopathologies in the interstitial tissue were fibrosis, disorganization, and proliferation of fibroblast-like cells (Fig. 6 (h)). All these interstitial changes may affect other cellular populations from the interstitium, mainly the Leydig cells, and seminiferous tubules as a secondary consequence. Leydig cells are important targets of high-dose Cd [56]. Our findings indicate that the morphological changes in the Leydig cells are correlated to high doses or increase in the time of Cd exposure, such as degenerative changes and appearance of tumors (Fig. 6 (i)). In addition, the interstitial injuries can affect the fluid movement inside the testis resulting in injuries at the seminiferous tubules since they are dependent on interstitial tissue homeostasis [45, 65]. As a result, the seminiferous tubules can be affected by the direct Cd impact and interstitial alterations.

Usually associated with interstitial injuries, our findings indicate that Cd exposure causes atrophy, degeneration, necrosis, and calcification of the seminiferous tubules. Necrosis and calcification/mineralization were the most common histopathological changes in the seminiferous tubules, besides germ cell death. Indeed, cell death and calcification are processes closely related, once increases in Ca^{+2} and inorganic phosphate in blebs (matrix vesicles) formed by apoptotic and/or necrotic cells are seemingly the primary mechanism of calcification. Additionally, membranous cellular degradation products resulting from cellular disintegration are often used as the nidus of this disorder [81]. The similarities between Cd^{+2} and Ca^{+2} allow Cd^{+2} to displace Ca^{+2} in some Ca^{+2}-binding proteins, and to disrupt Ca-mediated signaling pathways that lead to tissue damages [52]. Xie *et al.* [82] reported that Cd-induced cell death was mediated by the release of Ca^{+2} from intracellular Cd storage, which confirmed that the increased Ca^{+2} intracellular concentration is related to cell death (Fig. 6 (j)).

Although the main histomorphological tool used by studies for understanding the Cd impact on testicular tissue was qualitative analysis, some records, mainly of this century, confirmed the injuries by quantitative tools. The qualitative evaluation of the testicular parenchyma allows the visualization of histomorphological injuries in the tissue caused by Cd, showing cell and tissue modifications that are probably responsible for the organ malfunction [30]. On the other hand, the use of quantitative tools and morphometric and stereological methods is very desirable because they are very sensitive instruments to detect non-evident alterations, which could not be confirmed merely by the observation of the testicular morphology [45, 83]. Indeed, all methods are fundamental for the searches, so relying on only one type of data (i.e., number or words) is extremely limiting. By using qualitative and quantitative techniques within the same framework, researchers can incorporate the strengths of both methodologies, adopting the principle of complementarity [84, 85].

Strength and Limitations of the Current Review

Recently, studies about the exposure to environmental contaminants and male reproductive health are on the rise. However, no systematic review has been reported to investigate cadmium exposure and testicular tissue outcomes. The main strength of this study is its novelty and the applied findings that can be useful to provide a direction for future researches in this field and the development of decision making for therapeutic alternatives. Also, the AOP network of this study may help to understand the main mechanisms involved in the alterations triggered by other environmental contaminants, and, consequently, the translation to the human health risk assessment.

This review also has some limitations. The bias analysis demonstrated that fundamental characteristics, such as random sequence generation or random outcome assessment and blinding of participants (caregivers and outcome assessor), were not reported in the studies. In addition, some records provided incomplete outcome data and insufficient information about the control group, which affect the accuracy of the results. Overall, the evidence of the individual studies showed wide heterogeneity and so it was not possible to compare the data statistically. This kind of comparison should be avoided because it generates evidence that presupposes an apparent external validity (generalizability), which is in fact not supported by the available data set. In this sense, we identified that each study presented marked differences regarding the experimental model and methods of data collection, analysis, and interpretation, as well as the accuracy of the scientific report. In individual studies, each element of methodological bias is associated with some degree of variability in the research outcomes, with a direct impact on the quality of evidence. However, it is important to emphasize that all types of review have limitations and these limitations are more evident in systematic review studies once flaws methodological and

incomplete reports can produce inaccurate and unreliable conclusions. In our case, the major limitation was the heterogeneity of the studies, which makes it an arduous task to compare them. Therefore, considering these analytical limitations, we developed a systematic review admitting its intrinsic qualitative nature by describing important points of bias. We hope to contribute to future studies on avoiding those elements of bias that impair the quality of evidence.

Conclusions

Our results support that the Cd exposure induces significant histopathologies in the testis of all murine models regarding routes, in a dose- and time-dependent manner, in which damages, even some expected only in the chronic phase (e.g., degeneration and necrosis), can be observed during the first hours of exposure. These results allowed us to conclude that the mechanisms involved in the histopathological process start in the acute phase and are increased and enhanced during the chronic phase.

The AOP shows that the main mechanisms involved in Cd-induced histomorphologies may be related to three potential MIE: mimicry and interference of Cd with essential elements; change in the activity of oxidative phosphorylation enzymes; and gene expression alteration. The first cited MIE disrupts directly the endothelial structural proteins, in which the primary mechanism involved in the lesion may be vascular damage. All the MIE contribute to the increase in free radicals and consequently increase in ROS production, which leads to oxidative stress in the tissue with alterations in the antioxidant enzyme activities and, consequently, several injuries in the testis. Overall, our findings provide new insights into mechanisms of Cd-induced testicular toxicity.

Data Availability Not applicable.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Code availability Not applicable.

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