IARA MAGALHÃES RIBEIRO

EXPOSIÇÃO A METAIS AFETA PARÂMETROS ESPERMÁTICOS EM RUMINANTES DOMÉSTICOS? UM ESTUDO DE META-ANÁLISE

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Medicina Veterinária, para obtenção do título de *Magister Scientiae*.

Orientadora: Mariana Machado Neves

VIÇOSA - MINAS GERAIS
2021

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T
R484e
2021

Ribeiro, Iara Magalhães, 1992-
Exposição a metais afeta parâmetros espermáticos em ruminantes domésticos? : um estudo de meta-análise / Iara Magalhães Ribeiro. – Viçosa, MG, 2021.
55 f. : il. ; 29 cm.

Orientador: Mariana Machado Neves.
Dissertação (mestrado) - Universidade Federal de Viçosa.
Referências bibliográficas: f. 36-50.

1. Ruminantes - Espermatozoides. 2. Metais pesados. 3. Sêmen. 4. Toxicologia. I. Universidade Federal de Viçosa. Departamento de Biologia Geral. Programa de Pós-Graduação em Medicina Veterinária. II. Título.

CDD 22. ed. 636.082

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APROVADA: 26 de fevereiro de 2021.

Assentimento:

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Orientadora

Aos meus pais e irmãos.

AGRADECIMENTOS

A Deus, eterno protetor, pela vida, pela saúde, e por guiar meus passos permitindo que eu atingisse mais este objetivo.

À minha família: minha mãe, Maria Beatriz, por todo o amor, por todas as orações, e por nunca medir esforços para me proporcionar sempre o melhor; meu pai, Avimar Luiz (*in memoriam*) que, mesmo distante, se faz presente todos os dias guiando e iluminando o meu caminho; meus irmãos Alessandra, Bruna, e Marcos Vinícius, por serem meu alicerce; meus sobrinhos Cauã e Eduardo, por serem luz e fonte de alegria na minha vida; minha avó Dorvalina (*in memoriam*), pelas infinitas orações que abençoaram meu caminho para que eu o trilhasse sem medo; minhas tias Regina, Ana Maria, Marina, Raquel e Silvana; tios, primos e primas, em especial Júlia Maria, por todo carinho e acolhimento.

Aos amigos do LARAT, em especial Arabela e Renner, pelo apoio incondicional e pelos intermináveis exemplos de dedicação, caráter e companheirismo.

A todos os meus amigos, em especial Jéssica Anacleto, Janaína Gomes, Victoria Kanadani, Rachel Tavares, Denise Okano, Paula Maitan, e minha sogra Fernanda, por vibrarem comigo a cada conquista.

À minha professora e amiga Bruna Waddington de Freitas, pela ajuda e companheirismo excepcionais, por ser proteção, exemplo, e inspiração e por me ensinar, além das salas de aulas, muito mais sobre a vida.

Ao meu namorado Thiago, por sempre acreditar em mim e pelos incentivos que me ajudaram a chegar até aqui; pelas risadas, pelo amor e carinho imensuráveis.

À minha orientadora Mariana Machado Neves, pela participação ímpar no desenvolvimento e execução deste trabalho, pelo conhecimento compartilhado, pela dedicação, compreensão, paciência, e por sempre ter uma palavra de conforto nos momentos de incerteza.

À Universidade Federal de Viçosa e ao Programa de Pós-graduação em Medicina Veterinária pela oportunidade de realização do presente trabalho.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela concessão da bolsa de estudos.

A todos com que tive a oportunidade de conviver nestes últimos anos e que muito contribuíram para que eu pudesse chegar até aqui. Muito obrigada por tudo!

“Não sabendo que era impossível, foi lá e fez”.
(Jean Cocteau)

RESUMO

RIBEIRO, Iara Magalhães, M.Sc., Universidade Federal de Viçosa, fevereiro de 2021. **Exposição a metais afeta parâmetros espermáticos em ruminantes domésticos? Um estudo de meta-análise.** Orientadora: Mariana Machado Neves.

Durante a última década, os tóxicos ambientais foram sugeridos serem potenciais fatores de risco para a infertilidade masculina. Ao contrário dos poluentes orgânicos, os metais não são facilmente degradados e se acumulam ao longo da cadeia alimentar, tornando-se uma das principais causas da redução da qualidade seminal em animais. No entanto, ainda não está claro se os efeitos desses contaminantes são negativos, neutros ou positivos para as células espermáticas. Portanto, uma revisão meta-analítica foi conduzida para determinar se a exposição a doze metais afeta os parâmetros espermáticos de ruminantes domésticos. O modelo de efeitos aleatórios revelou que os metais provocaram um efeito forte e negativo na viabilidade espermática, um efeito moderado e negativo em parâmetros andrológicos e na motilidade espermática, e um efeito forte positivo na produção de metabólitos oxidativos. Por outro lado, a exposição aos metais não afetou a defesa antioxidante, níveis hormonais, concentração espermática e a fertilidade dos espermatozoides. Em geral, os mecanismos toxicológicos dos metais estão relacionados à sua capacidade de gerar estresse oxidativo, ligar-se a grupos tiol de proteínas e mimetizar minerais essenciais. O modelo de efeitos mistos mostrou que todos os moderadores metodológicos, como o tipo de metal, concentração de metal administrada, via de exposição, frequência de dosagem e modelo animal influenciaram de forma diferente os parâmetros espermáticos, considerando a variabilidade dos modelos. Em conclusão, nossos achados indicam que a exposição aos metais causa efeitos nocivos ao sistema reprodutor masculino ao inibir os parâmetros funcionais dos espermatozoides, importantes para uma fertilização bem-sucedida. Além disso, devido à característica ubíqua dos metais e sua alta toxicidade, esta revisão sugere que tais elementos podem contribuir para causas subjacentes de infertilidade. Além disso, este estudo pode direcionar pesquisas futuras sobre as consequências da contaminação por metais na fertilização e no desenvolvimento do embrião pré-implantação.

Palavras-chave: Espermatozoides. Metais pesados. Microminerais. Sêmen. Toxicologia.

ABSTRACT

RIBEIRO, Iara Magalhães, M.Sc., Universidade Federal de Viçosa, February, 2021. **Could metal exposure affect sperm parameters of domestic ruminants? A meta-analysis review.** Adviser: Mariana Machado Neves.

During the last decade, environmental toxicants have been suggested to be potential risk factors for male infertility. Unlike organic pollutants, metals are not easily degraded and accumulate along the food chain, making it a major cause of declined semen quality in animals. However, it is still unclear whether the effects of these contaminants are negative, neutral, or positive for sperm cells. Therefore, a meta-analytical review was conducted to determine whether the exposure to 12 metals affect the spermatozoa parameters and overall reproductive health of domestic ruminants. The random-effects model revealed that metals elicited a strong and negative effect on sperm viability, a moderate and negative effect on andrological parameters and sperm motility, and a strong positive effect on oxidative metabolites production. By contrast, metal exposure did not affect antioxidant defense, hormone levels, sperm concentration, and sperm fertility. Overall, the toxicological mechanisms of metals are related to their ability to generate oxidative stress, bind with thiol groups of proteins, and mimicry essential minerals. The mixed-effects model showed that all methodological moderators such as type of metal, metal concentration, exposure route, dosing frequency, and animal model differently influenced the spermatozoa parameters, accounting for variability in the models. In conclusion, our findings indicate that exposure to metals cause noxious effects to male reproductive system by inhibiting functional sperm parameters, important for a successful fertilization. Also, due to the ubiquitous characteristic of metals and its high toxicity, this review suggests that such elements may contribute to underlying causes of infertility. Furthermore, this study can direct future researches on the consequences of metal contamination on fertilization and pre implantation embryo development.

Keywords: Spermatozoa. Heavy metals. Microminerals. Semen. Toxicology.

SUMÁRIO

Could metal exposure affect sperm parameters of domestic ruminants? A meta-analysis review

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Running title: Metals and reproductive health of ruminants

Abstract

During the last decade, environmental toxicants have been suggested to be potential risk factors for male infertility. Unlike organic pollutants, metals are not easily degraded and accumulate along the food chain, making it a major cause of declined semen quality in animals. However, it is still unclear whether the effects of these contaminants are negative, neutral, or positive for sperm cells. Therefore, a meta-analytical review was conducted to determine whether the exposure to 12 metals affect the spermatozoa parameters and overall reproductive health of domestic ruminants. The random-effects model revealed that metals elicited a strong and negative effect on sperm viability, a moderate and negative effect on andrological parameters and sperm motility, and a strong positive effect on oxidative metabolites production. By contrast, metal exposure did not affect antioxidant defense, hormone levels, sperm concentration, and sperm fertility. Overall, the toxicological mechanisms of metals are related to their ability to generate oxidative stress, bind with thiol groups of proteins, and mimicry essential minerals. The mixed-effects model showed that all methodological moderators such as type of metal, metal concentration, exposure route, dosing frequency, and animal model differently influenced the spermatozoa parameters, accounting for variability in the models. In conclusion, our findings indicate that exposure to metals cause noxious effects to male reproductive system by inhibiting functional sperm parameters, important for a successful fertilization. Also, due to the ubiquitous characteristic of metals and its high toxicity, this review suggests that such elements may contribute to underlying causes of infertility. Furthermore, this study can direct future researches on the consequences of metal contamination on fertilization and pre implantation embryo development.

Keywords: Spermatozoa; heavy metals; microminerals; semen; toxicology.

1. Introduction

Environmental pollution is one of the most serious global health concerns (Anirudhan & Sreekumari, 2011; Rehman et al., 2018). Humans and animals are exposed daily to various chemicals and metals through several contaminated sources. Particularly, inorganic metals found in nature can become more available and accumulate in plant and animals as a consequence of anthropic activities, such as agriculture, mining, smelting, refining, and incineration of urban and industrial waste (Tavares & Carvalho, 1992). Altogether, these factors may influence the impact of metal exposure on livestock (Leita et al., 1991; Souza et al., 2009). For instance, plants can accumulate metals in their tissue and promote metal transfer through the food chain (Maiga et al., 2005). Large quantities of concentrates and mineral supplements are used to supply the productive demand of domestic animals (Abdalla et al., 2008; Da Silva et al., 2008). These nutritional supplements, obtained from industries without any bromatological or physio-chemical analysis, may have high concentrations of metals (Silva et al., 2007). It increases the susceptibility to metal intoxication, directly affecting the metabolism and reproductive performance of animals (Alkmim Filho, 2011; Costa et al., 2020).

Notwithstanding, livestock health is susceptible to the impact of catastrophes caused by human. For example, Brazil has faced two big mining disasters involving iron tailing rupture in 2015 and 2019, which spread millions cubic meters of tailing into Doce and Paraopeba rivers and their surrounding areas, causing devastation along its way (Quadra et al., 2019; Vergilio et al., 2020). In the end, the mud wave affected more than 41 cities (Davila et al., 2020; Girotto et al., 2020) that were involved in farming and dairy production. Chemical analysis confirmed the occurrence of several elements in water, sediment, soil, and vegetation from areas affected by Mariana and Brumadinho disasters, such as aluminum (Al), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), iron (Fe), manganese (Mn), mercury (Hg), nickel, (Ni), and zinc (Zn) (Thompson et al., 2020; Vergilio et al., 2020).

Deleterious effects of metal exposure rely on the type of metal, exposure route, dosing frequency, exposure time, and concentrations (Hardneck et al., 2018). Other factors such as species, age, genetic and nutritional factors need attention concerning ruminants (Avila et al., 2003). Heavy metals as Cd, Pb, Hg, and the metalloid arsenic (As) are known as relevant toxic metals. They do not exert physiological functions in living organisms and, even in low quantities, they are harmful and toxic to body systems, including reproductive tract (Flora et al., 2011; Renu et al., 2018; Mohammadi et al., 2020). On the other hand, other metals, such as cobalt (Co), Cu, Fe, Mn, magnesium (Mg), and Zn, have physiological functions in the body (Lapointe et al., 1996; Hernández-Meléndez et al., 2015; Arangasamy et al., 2018b, 2018c). They are co-factors of various enzymes in major metabolic pathways and are indispensable for physiological reproductive function (Mertz, 1981; Kendall et al., 2001). Despite they are not toxic per se, they can cause physiological disturbance when their concentration exceeds a certain threshold (Appenroth, 2010; Knazicka et al., 2012; Roy et al., 2014a), reducing testis functionality and impairing the sperm count, quality, and motility (Danadevi et al., 2003; Yousef et al., 2006; Roy et al., 2014b; Leahy et al., 2016). In fact, spermatozoa are susceptible to toxic metals due to their main three mechanisms of action: (1) excessive production of reactive oxygen species (ROS) with loss of oxidative defense capabilities, leading to oxidative stress; (2) binding to thiol/sulfhydryl groups of proteins, as well as (3) mimicry and displacement of important minerals such as zinc and calcium, interfere with its migration in sperm cells (Tchounwou et al., 2012; de Angelis et al., 2017).

Thus, understanding the disorders these metals may cause, as well as the molecular and biochemical mechanisms involved in these changes, can provide useful information about the potential impact on male reproductive health. Nevertheless, the number of published articles evaluating the effect of metals on spermatozoa of domestic ruminants is very scarce. Therefore, this review aimed to use a meta-analytical approach to determine the effect of Al, As, Cd, Cr,

Co, Cu, Fe, Pb, Mg, Mn, Hg, and Ni exposure on reproductive parameters of domestic ruminants, with focus on spermatozoa. This review also determined the magnitude of those effects and measure the influence of methodological moderators on the variables analyzed. The findings are expected to reveal the potential damage of these toxic chemicals on sperm parameters, with possible consequences to male fertility.

2. Methods

2.1.Search strategy

This study was conducted in accordance with the Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati et al., 2009). The search strategy for the present meta-analysis was designed to identify studies evaluating the effects of twelve metals (Al, As, Cd, Cr, Co, Cu, Fe, Pb, Mg, Mn, Hg, and Ni) on spermatozoa. The review included all studies published up to 2020. To perform the meta-analysis, an extensive bibliography search was conducted using the electronic databases Medline/PubMed, Web of Science, and Scopus, completed on February, 2020. For all databases, the searches were performed separately for each one of the twelve metals along with the following terms: 'sperm*' OR 'semen'. The wildcard symbol '*' was used to expand the search. In the case of Pb, the keywords 'lead acetate' and 'lead chloride' were used to reduce the number of irrelevant articles. Similarly, the keywords 'magnesium chloride', 'magnesium citrate', 'magnesium sulfate', and 'magnesium acetate' were used to expand the search field for Mg. When important studies were unavailable, there was an attempt to contact the corresponding author.

2.2. Selection criteria

To be considered eligible for this review, studies were required to meet the following criteria: (1) be published in a peer-reviewed journal; (2) used domestic ruminants (goat, ram, bull, and buffalo) as animal models; (3) presented control (without metal exposure) and treatment (with metal exposure) groups, for experimental studies; and (4) reported means, sample size, and a measure of variance (standard deviation or standard error for both the control and treatment groups). Ruminants were chosen as animal model here because they have similar semen features concerning sperm concentration, ejaculate volume, and mass motility (Salamon and Maxwell, 2000; Jiménez-Rabadán et al., 2016). Studies were excluded for the following reasons: (1) the study used females to perform the animal exposure or monogastric animals; (2) the study was a review article, letter to the editor, case study, comment, or editorial; (3) data content was not applicable or statistics were not reported or not adequate; and (4) full-text articles were unavailable. Two independent reviewers screened the search results to assess whether they met the selection criteria, with disagreement resolved with a third reviewer. Response mean values ($X_{control}$ and $X_{treatment}$), standard deviations ($S_{control}$ and $S_{treatment}$), and sample sizes ($N_{control}$ and $N_{treatment}$) were collected from the text, tables, and/or figures from each study included in this review. When data were available in figures, these were digitized, and means and measurements of variance were obtained using the software ImageJ® after calibrating each picture to the nearest 0.01 mm. Measurements of variance were all converted to standard deviations of the mean using MetaWin Statistical Calculator (Rosenberg et al. 2000).

A separate random-model effect of meta-analysis was conducted for each one of the following six variables related to sperm parameters affected by metals: (1) sperm viability, (2) andrological parameters, (3) sperm motility, (4) oxidative metabolites production, (5) antioxidant defense, (6) hormone levels, (7) sperm concentration, and (8) sperm fertility.

Hormone levels and andrological parameters were considered in this meta-analytical study once they are indirectly associated with sperm production. The parameters included in each variable are shown in Table 1.

Table 1. Parameters collected from the eligible studies and grouped into eight variables analyzed under random-effects model for meta-analysis review.

Variables	**Parameters**
Sperm viability	Normal sperm morphology, viability, HOST reactive cells, acrosome integrity, sperm membranes integrity, DNA integrity, mitochondrial membrane potential
Andrological parameters	Semen volume, testicular weight, length, width, and scrotal circumference
Sperm motility	Subjected evaluations under phase contrast microscopy and computer-assisted semen analysis
Oxidative metabolites	Concentration of malondialdehyde, protein carbonyls, hydrogen peroxide, reactive oxygen species, and superoxide anion generation
Antioxidant defense	Total antioxidant capacity, superoxide dismutase, catalase, glutathione S-transferase and glutathione peroxidase, reduced glutathione, myeloperoxidases, nuclear factor erythroid 2-like 2
Hormone levels	Testosterone, FSH, LH
Sperm concentration	Sperm count, sperm concentration
Sperm fertility	Cyclic AMP concentration, Ca^{+} concentration, transcript expression of genes related to fertility, *in vitro* fertilization, sperm zona binding, nuclear chromatin stability, acrosin proteolytic activity, capacitation patterns

AMP: adenosine monophosphate; Ca^{+}: calcium ion. DNA: deoxyribonucleic acid; FSH: follicle-stimulating hormone; HOST: Hypo-osmotic swelling test; LH: luteinizing hormone.

Only variables that generated at least five independent comparisons were included in the analysis. According to the information provided by the authors, a mixed-effects model of meta-analysis was performed by categorizing the studies according to five methodological moderators: type of metal, metal concentration, exposure route, dosing frequency, and animal model. These categories are presented in Table 2.

Table 2. Methodological moderators categorized according to the variables collected from the eligible studies included in the mixed-effects model of meta-analysis review.

	Moderators				
	Type of metal	Metal concentration (mg/L)	Exposure route	Dosing frequency	Animal model
Categories	Arsenic Cadmium Copper Iron Mercury	Up to 1.99 5 to 65 More than 80	Oral route *In vitro* incubation	Single dose Daily	Bull Buffalo Goat Ram

2.3. Meta-analysis

The standardized difference between the control and the treatment group was used to interpret and summarize the effects of metals/metalloids on male gamete and reproductive parameters. For each study, the magnitude of effect (d) was calculated as $d = (X_t - X_c / SD) * J$, where X_t is the response of the treatment group, X_c is the response of the control group, SD is the pooled standard deviation, and J is the correction term to remove bias towards small sample sizes (Rosenberg et al., 2000). J reaches 1.0 when the sample sizes ≥ 25.

After the calculation of Hedge's d for each independent comparison, the cumulative effect (d_{++}) for each of the variables surveyed was calculated using a random-effect model. This review also used mixed-effects models for the analysis of the moderators, as these models assume that the differences between studies within a class are determined by sampling errors and random variation. Upper and lower confidence intervals (CIs) were calculated according to the average cumulative effect, and intervals that did not overlap with zero, with n – 1 degree of freedom (df), were considered significant. By convention, a d_{++} values around 0.2 is considered to indicate a weak effect, 0.5 is considered to indicate a moderate effect, and 0.8 is considered to indicate a strong effect (Cohen, 1992). Additionally, positive d_{++} values indicate that the

chemicals have a positive effect on the variables measured, whereas negative values indicate a negative influence of the chemicals on the measured effects.

Finally, heterogeneity analyses (Q statistic) were employed to test whether categorical groups in mixed models were homogeneous concerning the calculated effect sizes. The total heterogeneity (Qt) for all effects tested and the heterogeneity within (Q_w) and between groups (Q_B) were calculated. The significance of these statistics was evaluated according to a chi-square distribution with n - 1 df. Because the analyses were based on only published studies, and studies showing large and significant effects may be more likely to be published than studies that show weak or no effects (the 'file-drawer problem', *sensu* Rosenthal 1979), fail-safe numbers were calculated for each effect tested. Fail-safe numbers indicate the number of non-significant, unpublished, or missing studies that would need to be added to the sample to change its results from significant to non-significant (Rosenberg et al. 2000). As a rule of thumb, fail-safe results are considered robust if the fail-safe number exceeds $5k + 10$, where k is the number of comparisons in the analysis. All analyses were conducted using MetaWin 2.1 (Rosenberg et al. 2000), and figures were made using Sigma Plot 10.0 software.

3. Results

3.1. Literature search and study selection

The initial search resulted in a total of 12.839 records identified through database searches in Medline/PubMed, Web of Science, and Scopus. Following the removal of duplicates and exclusions, 36 studies were eligible for this meta-analysis study: As (n = 4), Cd (n = 8), Cu (n = 13), Fe (n = 2), Mn (n = 4), Hg (n = 5). Two studies were included in more than one metal. Figures 1 show the diagram of the articles search and selection process for each metal. Studies identified for the remaining metals, Al, Co, Cr, Pb, Mg, and Ni, did not meet the

inclusion criteria of the current meta-analysis, and were not analyzed. The reasons for their exclusion are shown in Supplementary Table 1.

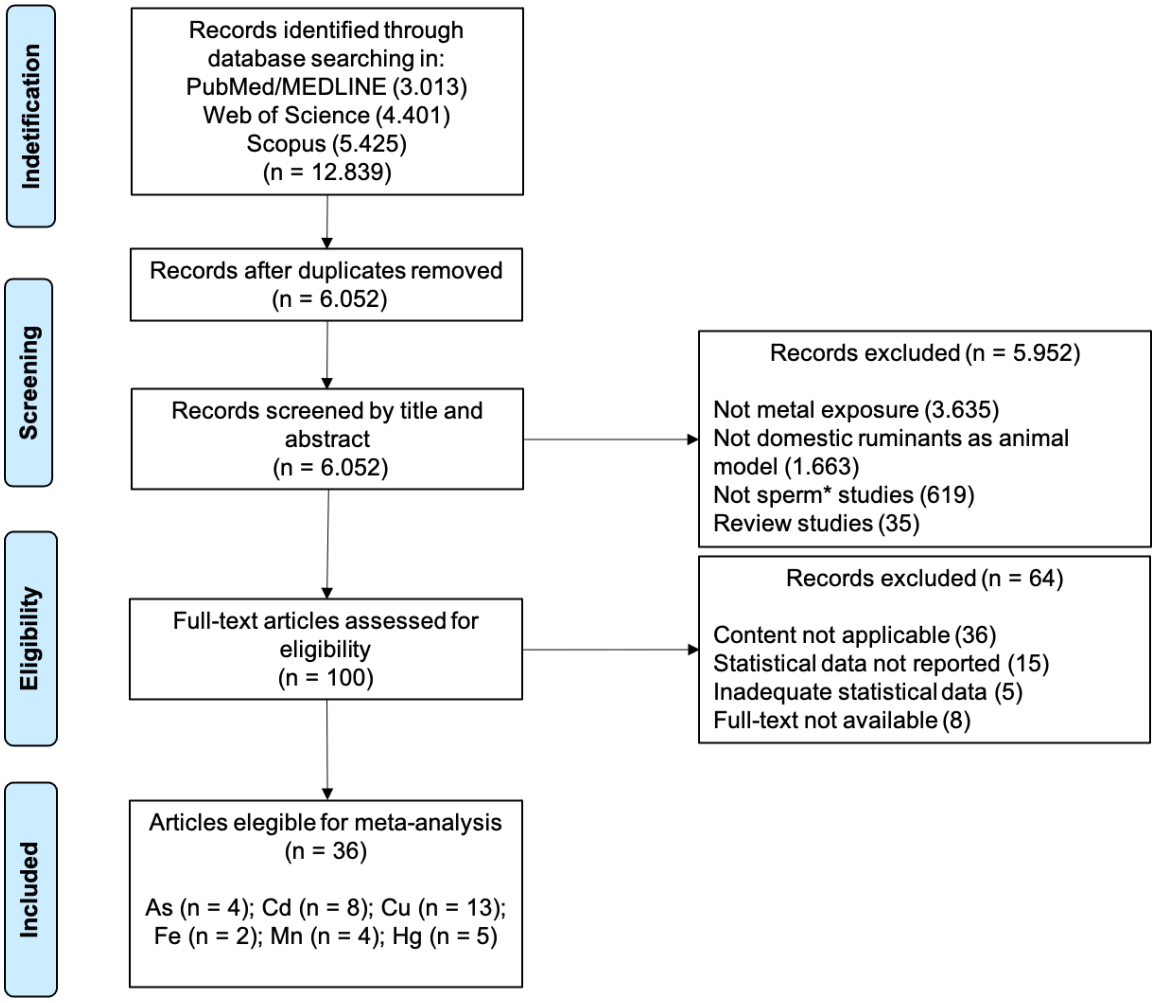

Fig. 1. Search study and selection process to As, Cd, Cu, Fe, Mn, and Hg articles. (n) = number of studies.

3.2. Qualitative data

From those 36 studies, we have got 212 independent comparisons for the effects of metals on spermatozoa parameters of ruminants. Among them, 14 studies were conducted in bulls, 12 in goats, eight in rams, and two in buffalos. Overall, 24 studies were performed *in*

vitro, administered as single doses, and 12 was performed *in vivo*, orally in daily doses. Further, 12 studies tested the effects of metals at concentrations up to 1.99 mg/L, 14 used concentrations between 5 and 65 mg/L, and 11 tested concentrations higher than 80 mg/L. One study tested more than one concentration (Roy et al., 2014a). Twenty-four studies analyzed the effects of metals for a period of time up to 1 day, five between 7 and 84, and ten tested exposure for a period longer than 100 days. From those, two studies were included in more than one of these categories (Lymberopoulos et al., 2003; Arangasamy et al., 2018a). The majority of studies (32) used ejaculated semen, which were collected using artificial vagina (21) and electro-ejaculator (11). The other four studies collected epididymal sperm by sperm washing. Supplementary Table 2 shows detailed qualitative data of each study included.

3.3. Meta-analysis

Metal exposure elicited a strong and negative effect on sperm viability, and a moderate and negative effect on sperm motility (Fig. 4). Moreover, exposure to these elements exhibited a strong and positive effect on the production of oxidative metabolites (Fig. 4). Rosenthal's fail-safe number for these effects were fairly high relative to the number of independent comparisons (Sperm viability: 984.9; Sperm motility: 3,196.9; oxidative metabolites: 288.9). Although the variable andrological parameters showed a moderate and negative influence of metal exposure, it showed a low Rosenthal's fail-safe number (27.9). By contrast, metal exposure did not affect neither antioxidant defense, hormone levels, sperm number, and sperm fertility ($P > 0.05$; Fig. 4).

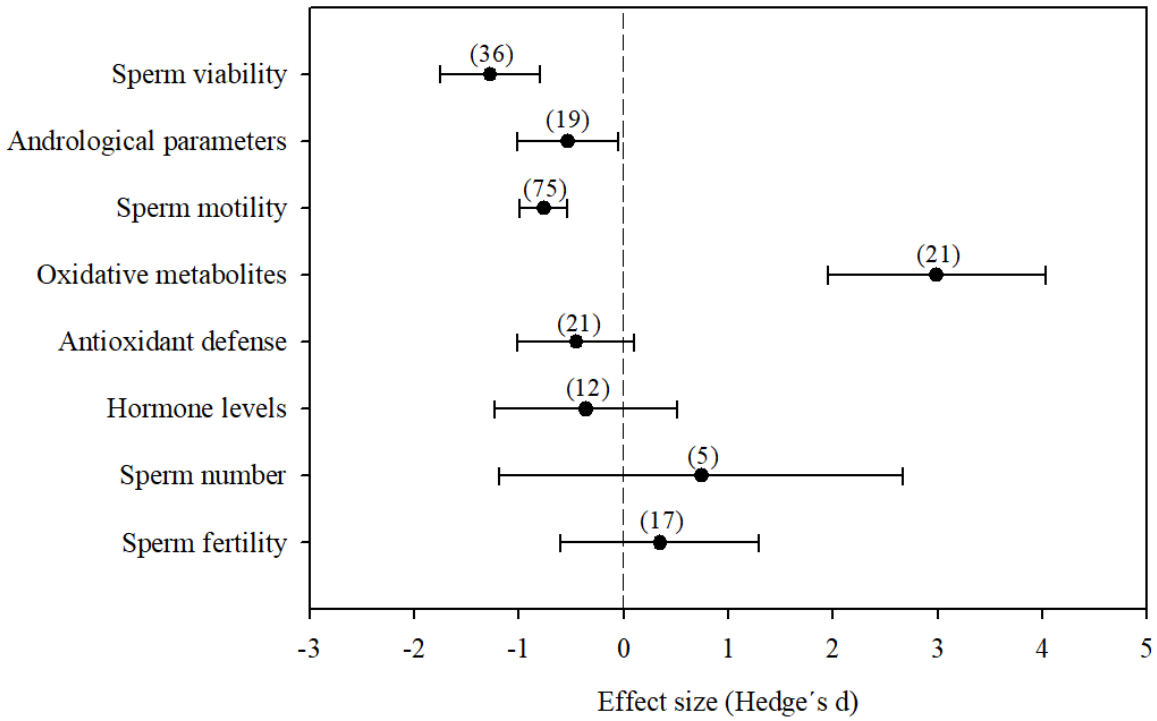

Fig. 2. Effects of As, Cd, Cu, Fe, Mn and Hg on spermatozoa and reproductive parameters analyzed. The cumulative effect size is reported with its 95% confidence interval and effects are significant if confidence intervals do not overlap with zero. (n) = number of independent comparisons.

3.4. Methodological moderators

Only variables statistically significant on random-effect model were analyzed by the methodological moderators. In this sense, the mixed-effects model revealed that the type of metal, and metal concentration influenced the variables androlgical parameters, sperm motility and viability, as well as oxidative metabolites production. Dosing frequency, exposure route, and animal model, in turn, influenced all of those variables, except androlgical parameters.

With respect to the type of metal, As exerted the strongest and negative effect on androlgical parameters and sperm viability (Fig. 5). On the contrary, Cu showed a moderate and positive effect on sperm viability. Sperm motility, in turn, was mainly affected after Fe

exposure when compared to Cd and Hg (Fig. 5). The exposure to Hg elicited the strongest and positive response in oxidative metabolites production (Fig. 5). Moreover, metal concentrations between 5 and 65 mg/L elicited a stronger and negative effect on andrological parameters, sperm motility, and sperm viability. The latter variable, additionally, exhibited a moderate and positive influence after exposure to metals at concentrations > 80 (Fig. 5). Oxidative metabolites exhibited a strong and positive influence under exposure to concentrations < 1.99 mg/L.

Furthermore, *in vitro* sperm incubation with metals caused the strongest and negative effects on sperm motility and viability, and the strongest and positive effect on oxidative metabolites production compared to oral route (Fig. 6). Likewise, single dose exerted the strongest effects on those variables instead of daily doses (Fig. 6).

Buffalo was the animal model mainly affected negatively at a strong magnitude by metal exposure, followed by ram, when the variables sperm motility and viability were analyzed (Fig 7). On the other hand, metal exposure exhibited a stronger positive effect on oxidative metabolites production in bulls than goats (Fig. 7).

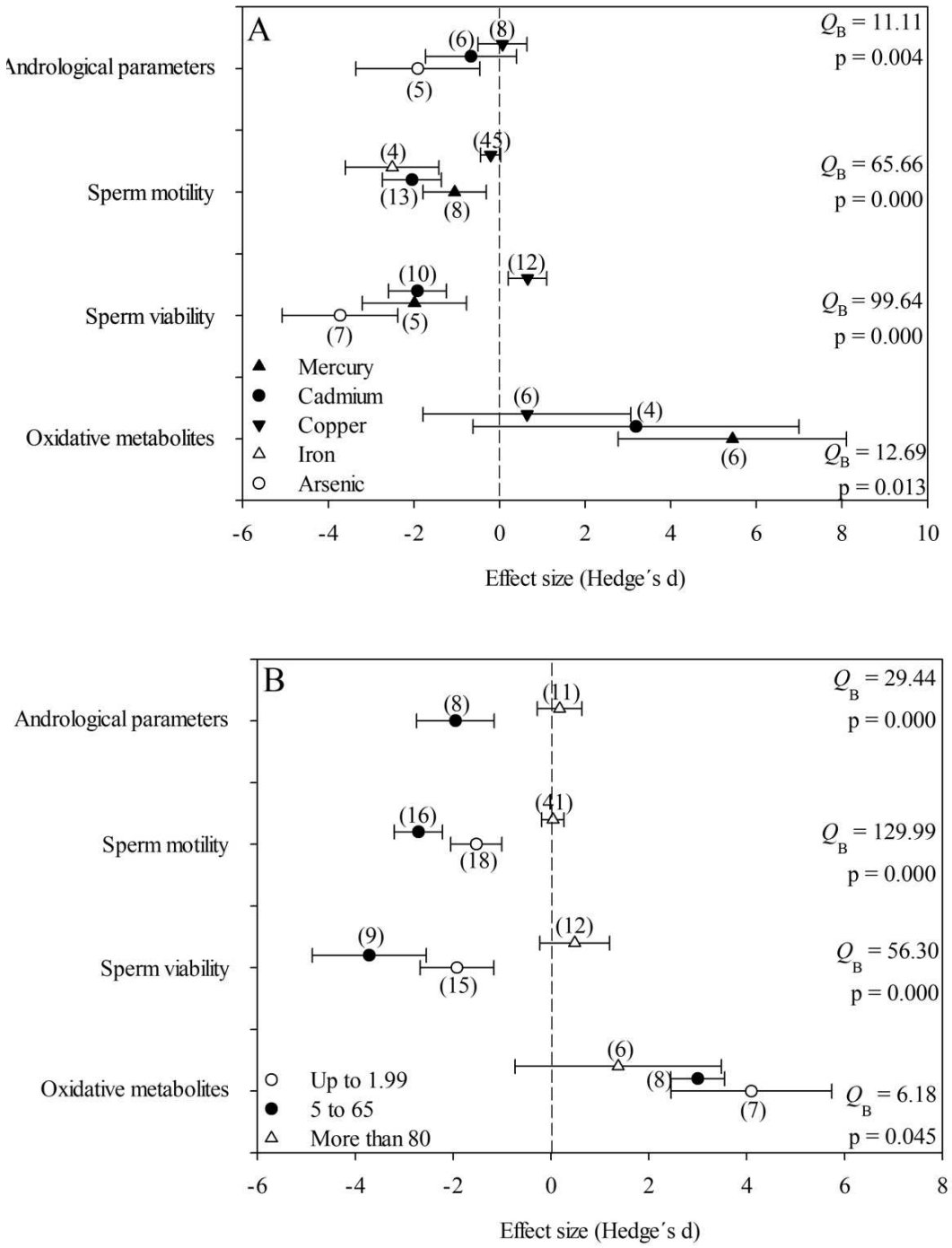

Fig. 3. Effects of type of metal (A) and metal concentration (B) on andrological parameters, sperm motility, sperm viability, and oxidative metabolites. The cumulative effect size is reported for each effect measured with its 95% confidence intervals, and effects are significant if confidence intervals do not overlap with zero. Numbers in parentheses indicate the number of independent comparisons for each effect. Q_B indicates heterogeneity between groups.

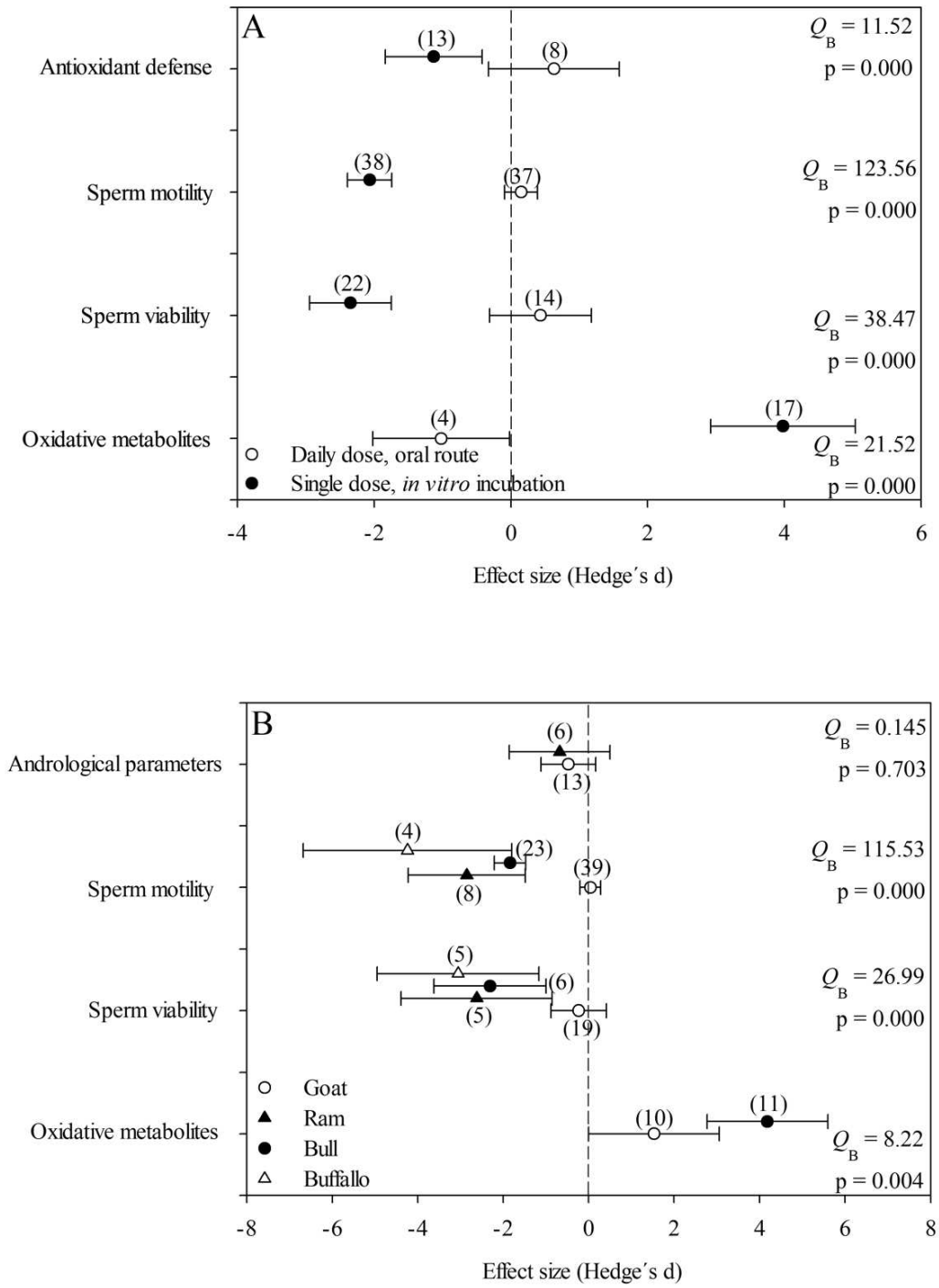

Fig. 4. Effects of exposure route/dosing frequency (A) and animal species (B) on antioxidant defense, sperm motility, sperm viability, oxidative metabolites, and andrological parameters. The cumulative effect size is reported for each effect measured with its 95% confidence intervals, and effects are significant if confidence intervals do not overlap with zero. Numbers in parentheses indicate the number of independent comparisons for each effect. Q_B indicates heterogeneity between groups.

4. Discussion

Overall**,** the findings of the random-effects model of meta-analysis indicate that exposure to metals negatively impact spermatozoa parameters of domestic ruminants. Herein, we observed that these toxicants elicit a strong and negative effect on sperm viability, a moderate negative effect on andrological parameters and sperm motility, and a strong positive effect on oxidative metabolites production. By contrast, metals did not exert significant difference on antioxidant defense, hormone levels, sperm number, and sperm fertility, revealing the scarcity of data in the literature. Metal pollution is one amongst the most serious environmental issues (Anirudhan & Sreekumari, 2011). These elements are directly or indirectly discharged into seawater and ecosystems, and have an impact on the aquatic environment and animal health (Tchounwou et al., 2012; Kumar et al., 2017; Fakhri et al., 2018). Heavy metals such as Cd, Cr, Pb, Hg and metalloids such as As, are typically toxic even at low concentrations, and non-biodegradable, generally resisting to conventional elimination treatments (Sall et al., 2020). The bioaccumulation and high reactivity of such elements make toxicity a serious public health problem. In Brazil, due to continued environmental disasters committed by mining companies, there are increasing concern about the harmful effects these chemicals can cause to human and animal health. Indeed, a large part of the livestock properties use superficial springs as a source of water, both for animals and for irrigation (Costa et al., 2020), increasing the susceptibility of domestic ruminants to poisoning. Several metals are considered reproductive toxicants and endocrine disruptors, due to impairment of hypothalamic-pituitary axis or by direct effect on Leydig and Sertoli cells, resulting in poor semen quality (Chowdhury, 2009; Mendiola et al., 2011; Pizent et al., 2012).

In this study, we showed that metals elicited a strong and negative effect on sperm viability. This variable indicate the functional quality of sperm to move toward an egg (Eskandari & Momeni, 2016b), and is known as a useful parameter to differentiate between

fertility and infertility conditions of males (Guzick et al., 2001). Therefore, attention to parameters estimating integrity of sperm or reflecting its metabolic state can provide information about the competence of sperm for fertilization process (Eskandari & Momeni, 2016a). The decrease in cell viability may be due to the increase in ROS production (Koizumi & Li, 1992; Figueiredo-Pereira et al., 1998) and decrease in cellular antioxidant defense systems, including catalase (CAT), glutathione (GSH), and GSH peroxidase (GPx) (Yang et al., 1996). A diminution of antioxidant reserves caused by toxic metals would increase the susceptibility of the sperm membrane to peroxidative injuries (Koizumi & Li, 1992), followed by changes in the membrane of cell organelles and loss of its functions. It can also cause a rapid decrease in mitochondrial membrane potential, change the activity of mitochondrial enzymes, cause significant changes in morphology, and disrupt the internal integrity of organs (De Vizcaya-Ruiz et al., 2009), consequently interfering with cell morphology percentage. Oxidative stress not only disrupts sperm's fertilization ability, but also reduces the integrity of sperm chromatin, leading to high frequency of single- and double-stranded DNA breaks (Aitken & Krausz, 2001; Saleh et al., 2003). Also, one possibility of reducing sperm integrity may be related to a decrease in ATP. Disruption of ATP supply through mitochondrial membrane disturbance is an important mechanism for decreased viability in sperm samples treated with metal ions (Au et al., 2000). Significant decrease in sperm viability seems to be mediated through compromised mitochondrial function due to depletion of ATP, cytochrome-c release and consequent activation of apoptotic cascade (Shenker et al., 2000).

Metals also exhibited a moderate negative effect on andrological parameters. Eliminating animals with low reproductive performance as early as possible is one of the main goals to be achieved in genetic selection programs. In this case, a thorough andrological examination should be performed to identify sub or infertile animals, as well as animals ready to breed (Henry et al., 2017). Andrological parameters such as semen volume, testicular weight,

length, width, and scrotal circumference of animals exposed to metals in this study was significantly reduced, which can be attributed to a reduction in testosterone levels, leading to subnormal androgenic stimulation or damage to accessory sex glands (Mann, 1964; Hogue et al., 1984), which are responsible for seminal plasma production (Ansa et al., 2017). The reduction of testosterone, in turn, may be due to the generation of ROS during mitochondrial respiration (Chen et al., 2005), as well as by the cytochrome P450 enzymes of the steroidogenic pathway in Leydig cells (Hornsby, 1989; Peltola et al., 1996), with consequent atrophy of the latter. Also, metal exposure can induce reduction of serum levels of FSH and LH, which may occur as a result of the high levels of glucocorticoids secreted by the adrenal glands (Bernstam & Nriagu, 2000). The increased concentration of corticosterone might inhibit the sensitizing activity of gonadotroph cells to GnRH, leading to the cessation of gonadotropin secretion (Kamel & Kubajak, 1987). Besides low semen volume, as a consequence of low testosterone concentration, reduced sperm production and concentration is likely to occur, once high level of testosterone is crucial for normal spermatogenesis (Anahara et al., 2006). Metals such as cadmium and arsenic have been reported to induce necrotic degeneration of the testis (Predes et al., 2010) and to destroy testicular germ cells due to seminiferous tubules damage (Zubair et al., 2016b), probably causing a cessation of spermatogenesis (Qadori & Al-shaikn, 2012), what may contribute to reduced testicular weight, length, width, and scrotal circumference.

Metals also elicited a moderate negative effect on sperm motility, which is considered to be one of the most important parameter of mature sperm (Alabi, 1985; Eskandari & Momeni, 2016a), obtaining a central role in the routine diagnosis of male fertility (Aitken, 1990). Previous published studies suggests that sperm motility may be one of the most sensitive parameters altered by metals exposure (Dawson et al., 1998; Xu et al., 2003). The mechanisms involved in the impairment of spermatozoa motility by such elements are multiple. Generally, when these metals are ingested through food or water, they are acidified and oxidized to their

oxidative states (As^{2+}, Cd^{2+}, Hg^{2+}, e.g.), which can readily bind to biological molecules, such as proteins and enzymes, and form stable and strong bonds (Chrestensen et al., 2000; Engwa et al., 2019). The most common functional group that metals bind is the thiol/sulfhydryl group (SH group of cysteine and SCH_3 group of methionine), which are located in proteins of flagellum and chromatin of sperm (Zubair et al., 2014). The sulfhydryl group of tubulins, the main component of sperm axonemal microtubules, disrupts the interaction between axonemal microtubular proteins and dynein motors, which are essential for sperm flagellar movement. Furthermore, accumulating evidence suggests that production of excessive ROS or depletion of oxidative defense capacity with consequent induction of oxidative stress plays a major role in mediating metal-induced cellular injuries (Kasprzak, 1995; Tchounwou et al., 2012). The plasma membranes of spermatozoa contain large amounts of polyunsaturated fatty acids (PUFA), which are particularly susceptible to damage from excessive ROS production, leading to generation of lipid peroxides and aldehydes. There is a significant negative correlation between lipid peroxidation (LPO) in the plasma membrane, the production of toxic aldehydes (such as MDA), and motility loss in caprine (Bucak et al., 2009), buffalo (Singh et al., 1989), rabbit (Alvarez & Storey, 1984) and boar (Cerolini et al., 2000) spermatozoa.

This meta-analytical review showed that metals elicited a strong positive effect on oxidative metabolites. Metal ions, as transition metals, cause cell damage by the formation of hydroxyl radicals (OH•), which are derived from superoxide anions and hydrogen peroxide under the Haber-Weiss reaction (Arabi, 2005, 2006). Hydroxyl radical is the most common free radical produced by metals oxidation, and can react with proteins, lipids and DNA, causing deleterious effects to them (Kerkeni et al., 2016). It is well established that metal-induced production of ROS can attack PUFAs, such as phospholipids present in sperm membranes, through LPO. The main aldehyde product of this process MDA, which is a marker of this process (Kerkeni et al., 2016). The increase in the formation of ROS leads to a reduction in

plasma membrane fluidity and structural integrity, which affects membrane transport and ultimately leads to immotile sperm (El Sisy et al., 2016). According to previous reports, the specific structure of sperm and its plasma membrane, damage to mitochondria, low cytoplasm and low antioxidants in sperm make these cells more susceptible to damage by free radicals (Jones et al., 1979; Aitken et al., 1989; Aitken, 1995; Amidi et al., 2016), which can cause DNA damage and reduce fertility (Chen et al., 1997). Fertilization, in turn, might be still feasible, since the oxidative damage of sperm DNA is not directly related to the reduction of sperm motility, which leads to subsequent detrimental consequences: increased embryonic death and malformation (Mao et al., 2018).

By contrast, antioxidant defense, hormone levels, sperm number, and sperm fertility were not significantly reduced by the metals. Although some individual studies indicate a decrease in hormone levels (Lymberopoulos et al., 2003; Zubair et al., 2016a; Arangasamy et al., 2018a; Mayasula et al., 2019), and sperm concentration (Lymberopoulos et al., 2000; Zubair et al., 2016a) by metals exposure, in the present review, those were insufficient to affirm such reduction is these variables. As hormonal level is an indirect parameter of sperm quality, only a few studies provided information about this variable here. The methodological variation may also have influenced, once some authors analyzed testosterone on seminal plasma (Mayasula et al., 2019) while others analyzed this hormone on blood plasma (Lymberopoulos et al., 2003; Zubair et al., 2016a; Arangasamy et al., 2018a). The non-significance of sperm number might also have been influenced by the sperm collection method. The majority of authors used electro-ejaculator (Lymberopoulos et al., 2000; Arangasamy et al., 2018a; Narasimhaiah et al., 2018; Mayasula et al., 2019), which is known to stimulate a greater release of seminal plasma, decreasing sperm concentration. Albeit sperm concentration is used routinely in the field, it is important to note that there are other parameters for sperm count evaluation, such as daily sperm production from testis or epididymal transit (Martínez et al., 1994; Sousa et al., 2014), providing

a more accurate analysis. Further, antioxidant defense was not significantly reduced by the metals, despite the negative effect exerted by Fe in bovine and buck spermatozoa (Tvrdá et al., 2015b) and Hg (Arabi, 2005; Da Silva et al., 2019; Kushawaha et al., 2020). This may be explained by the influence of Cu that, as an essential trace mineral, showed a positive influence in this variable, and might not promote an increase in ROS production strong enough to deteriorate the antioxidant balance. According to Dutta et al. (2019), the intracellular ROS concentration depends on the balance between the rate of ROS generation and its clearance through various antioxidant defense mechanisms. Thus, when the production of ROS does not exceed the antioxidant threshold, or the production of antioxidants is not reduced, oxidative stress might not occur. Indeed, Arangasamy et al. (2018c), Narasimhaiah et al. (2018) and Mayasula et al. (2020) reported that organic Cu supplemented goats exerted an opposite effect than that of Fe and Hg, increasing antioxidant enzyme activities and reducing oxidative stress. Finally, the prediction of *in vitro* fertilization ability of sperm cells must be carried out with caution, once this process is a chain of events, rather than an isolated phenomenon. A successful fertilization requires several attributes, and the interruption of any step may certainly cause fertilization failure. For instance, during fertilization, sperm should maintain functional membrane integrity for oocyte binding and acrosome reaction. It also involves an influx of extracellular calcium, increase in cyclic AMP, and decrease in intracellular pH. In addition, optimal fertility in males depends on whether the acrosome is structurally and biochemically intact, which contains the enzymes necessary to penetrate the outer layer of the egg and achieve fertilization (Selvaraju et al., 2011; Anchordoquy et al., 2019; Kushawaha et al., 2020). The few parameters included in this variable were analyzed individually and represents only part of the analyzes performed to clearly determine sperm fertility, influencing the non-significance of the results.

The mixed-effects model of meta-analysis revealed that the type of metal, metal concentration, animal model, exposure route and the dosing frequency influenced the variables analyzed. Arsenic elicited the strongest and negative effect on andrological parameters. Surprisingly, Cd did not exert statistical difference for this variable. There are no studies assessing the testicular damage or the susceptibility of spermatogenesis to both As and Cd in goats and rams. Nonetheless, in murine models, Cd is proved to cause detrimental damage to seminiferous tubules (Cupertino et al., 2017b; Da Silva et al., 2020; Mouro et al., 2020). Although As also damage the histological structure of testis (Awal et al., 2015), creating vacuoles within the seminiferous tubules (Zubair et al., 2020), the main effect of this metalloid on spermatogenesis is the disruption of the meiotic and post-meiotic stages of spermatogenesis (Li et al., 2015), reduction of the meiotic index (Couto-Santos et al., 2020), and impairment of the differentiation of spermatozoa from round to elongated spermatids (Li et al., 2015; Han et al., 2020). In relation to Cd, it induced testicular dystrophic calcification, in which the testicular capsule, tubular and intertubular compartments were targets of calcium deposition (Cupertino et al., 2017a). Cd was also reported to cause interstitial injuries such as hemorrhage, edema, fibrosis, disorganization (collagen fibers), necrosis, and inflammation (Massányi et al., 1996). In addition, Cd cause several changes in seminiferous tubules including necrosis, calcification/mineralization, and the presence of multinucleated giant cells (Cupertino et al., 2017a). One study have also confirmed that the damaged tubular area is replaced by fibrous connective tissue or amorphous mass (Da Silva et al., 2020). Altogether, these findings suggest that Cd causes a more severe damage to testicular histological architecture than As, with increased fibrosis, calcification, and edema, which might explain the increased testicular biometric parameters after Cd exposure.

Arsenic also elicited the strongest and negative effect on sperm viability than that of Cd and Hg. Some authors report that this metalloid stimulates an important disruption of the

electron transfer chain and subsequent generation of ROS, which leads to hydrogen abstraction from the PUFA-rich cell membrane of spermatozoa and the generation of cytotoxic aldehydes (Aitken et al., 2012), with consequent lipid peroxidation (Hosseini et al., 2013; Eskandari & Momeni 2016b). Once the cell has entered a state of oxidative state, activation of the intrinsic apoptotic cascade may occur, culminating with loss of sperm motility, vitality and DNA integrity. Although spermatozoa have a unique antioxidant matrix that can neutralize free radicals, this ability is lost once the antioxidant defenses become overwhelmed (Gibb et al., 2016), which occurs with As exposure (Souza et al., 2018, 2020). Thus, any slight increase in oxidative stress experienced by sperm cells may spread rapidly and cause deleterious damage to sperm motility, viability and fertility (Aitken et al., 2012; Aitken & Baker, 2008). Arsenic has also the ability to bind with SH group of proteins, thereby depleting the content of mitochondrial GSH, which is a necessary factor for maintaining the thiol group of mitochondrial proteins in a reduced state (Zhang et al., 2008). Under oxidative stress, thiol groups oxidation by As might lead to mitochondrial permeability transition pore dysfunction, leading to unlimited movement of protons into the mitochondria, and the subsequent collapse of mitochondrial membrane potential (Shen et al., 2000; Eskandari & Momeni 2016b).

Iron elicited a stronger and negative effect on sperm motility compared to Cd and Hg. Numerous studies have shown that promoter systems based on ferrous and ascorbate ions are extremely suitable for inducing oxidative stress in sperm cells (Baker & Aitken, 2005; Bansal & Bilaspuri, 2008). A gradual decline in bovine sperm motility and a simultaneous increase in superoxide concentration was observed in the experimental groups supplemented with high Fe doses (Tvrdá et al., 2015a, 2015b). This might be attributed to the critical oxidative stress on the sperm during *in vitro* culture (Aitken et al., 1993). The excessive production of ROS due to Fe overload has been shown to affect glyceraldehyde 3-phosphate dehydrogenase, a key enzyme of glycolytic process, resulting in a decrease in intracellular ATP levels, which

indicates that sperm motility and viability are gradually lost after oxidative damage (de Lamirande & Gagnon, 1992).

Mercury showed the strongest and positive effect on oxidative metabolites. The harmful effects of Hg are mainly due to its capacity to provide elevated amounts of free radicals by releasing accumulated lipid hydroperoxides from sperm membranes or by directly generating oxygen derivatives. This process might induce the peroxidation cascade, with consequent depletion of GSH content in many tissues and propagation of LPO process (De Flora et al., 1994), triggering the increased production of ROS. Exposing sperm cells to this oxidative environment significantly increases DNA damage (Arabi, 2005; Hansen et al., 2006; Martinez et al., 2016), once Hg has a high affinity for macromolecules and binds to DNA (Ariza & Williams, 1996) modifying all bases and generating base-free sites, deletions, defective frame shifts and DNA cross-links (Duru et al., 2000). In addition, Hg is capable of inhibiting antioxidants, such as glutathione peroxidase (GPx), glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) (Kalender et al., 2013).

Concentrations between 5 to 65 mg/L elicited the strongest negative effect on sperm motility, sperm viability and andrological parameters. This dosage is much higher than the maximum metal concentration tolerated in drinking water for As (0,01 mg/L), Cd (0,003 mg/L), Cu (2 mg/L), Fe (0,3 mg/L), Hg (0,006 mg/L) (World Health Organization [WHO] 2017). In addition, concentrations > 80 mg/L was not significantly different on these variables, except for sperm viability, once the metals may reach a threshold at a certain point, so that increasing concentrations might not change the response of metals. By contrast, this concentration increased sperm viability, probably due to the influence of Cu, which is an essential metal that stimulated a positive effect in this variable. Concentrations up to 1.99 mg/L showed a strong positive effect on oxidative metabolites, and this may be explained by the greater number of *in*

vitro studies performed with this dosage, which elicit a direct impact on sperm cells, increasing the magnitude of the effects.

In vitro exposure and metal administration in single doses elicited the strongest negative effect on antioxidant defense, sperm motility, viability and positive on oxidative metabolites. This kind of study was the experimental design mostly used to analyze the effect of metals on spermatozoa. In recent years, the use of *in vitro* systems in toxicology research has grown rapidly (Sandrini et al., 2009). This type of exposure may provide a direct and controlled environment that increases the magnitude of the effects, with basic information about the nature of the tested metal and/or cellular response (Binelli et al., 2009a). However, it occurs outside of living organisms, and they may not replicate the complexity of metabolism of an animal. On the other hand, *in vivo* experiments comprise the whole organism, so it can permit exploring the full effects of the toxicant without excluding any biochemical pathways (Binelli et al., 2009b). Similarly, single doses are usually administered in larger quantities for a short period of time, generating strong and acute effects. Daily doses are generally administered at lower concentrations for a longer time, resulting in subtle effects. These facts may suggest the strongest negative effect of *in vitro* exposure and single doses on sperm motility, sperm viability, and oxidative metabolites.

In relation to animal model, buffalo elicited a stronger and negative effect on sperm motility and sperm viability than ram and bulls. Buffalo sperm cells might be more susceptible to LPO and oxidative damage than that of cattle and rams, once its plasma membrane has higher quantity of PUFAs, such as arachidonic acid and docosahexaenoic acid (Singh et al., 1989). High concentrations of PUFA contribute to increased levels of fluidity and elasticity (Aurich et al., 2018). This makes sperm cells more susceptible to lipid membrane peroxidation, especially when combined with high concentrations of ROS (Aitken & Fisher, 1994). During LPO, cytotoxic aldehydes are produced, including acrolein, 4-hydroxynonenol (4HNE) and MDA.

Once produced, these cytotoxic aldehydes may further destroy electron transport chain by covalent bind to exposed proteins, thereby impairing sperm motility, membrane integrity and fertility (Aitken et al., 1993). On the other hand, bulls showed a stronger and positive effect on oxidative metabolites than goats. Studies using buffalos did not analyze the production of oxidative metabolites. There are no studies comparing the concentration of PUFA on sperm plasma membrane of bulls and goats. The cause of the higher positive effect of bulls on oxidative metabolites is unknown.

5. Review limitations

This review faced some limitations regarding methodological diversity. The eligible studies present relevant divergence in methodology, such as different animal models (goat, ram, bull, buffalo), and exposure route (*in vitro*, *in vivo*). Moreover, this review revealed the scarcity of studies evaluating spermatozoa parameters of domestic ruminants after metals exposure. For instance, this meta-analysis did not include results of Al, Co, Cr, Pb, Mg, or Ni. Even within the literature available, there is insufficient information of metals intoxication regarding methodological moderators, such as chemical form, and variables as sperm damage and number, what increases the confidence interval and Rosenthal's number. Also, as a consequence of scarcity of studies, the random-effect model could not be performed for each metal separately, probably masking and making it difficult to interpret the true impact of each metal on the different variables.

6. Conclusion

This meta-analysis provides the first compilation of data on the effects of metals intoxication on spermatozoa of domestic ruminants. We have summarized the impacts of As, Cd, Cu, Fe, and Hg on spermatozoa parameters, as well as their mechanisms of action. A negative effect of metals on sperm motility, viability, andrological parameters, and a positive effect on oxidative metabolites was certainly established. This outcome is probably driven by three main mechanisms: production of excessive ROS or depletion of oxidative defense capacity with consequent induction of oxidative stress; binding to thiol/sulfhydryl groups, and mimicry and displacement of essential minerals such as zinc and calcium, interfering with its migration in sperm cells during maturation. These findings elucidate the risks of these metals for domestic ruminants, since they are naturally present in the ecosystem and have the ability to bioaccumulate in the organism, causing deleterious effects in the reproductive system. Also, our findings indicate that exposure to metals cause deleterious effects to male reproductive system by inhibiting functional sperm parameters, important for successful fertilization. Therefore, due to the ubiquitous characteristic of metals and its high toxicity, this review suggests that such elements may contribute to underlying causes of infertility in farm animals. This study can direct future researches on the consequences of metal contamination on fertilization and pre implantation embryo development.

Supplementary data

Supplementary Tables 1- 2.

Declaration of interest statement

No conflict of interest to declare.

Funding

This research was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) [master fellowship to IMR], Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

7. References

Abdalla, A. L., Da Silva Filho, J. C., De Godoi, A. R., De Almeida Carmo, C., & De Paula Eduardo, J. L. (2008). Utilização de subprodutos da indústria de biodiesel na alimentação de ruminantes. *Revista Brasileira de Zootecnia*. https://doi.org/10.1590/S1516-35982008001300030

Aitken R. J, Clarkson J.S, Fishel S. (1989). Generation of reactive oxygen species, lipid peroxidation and human sperm function. *Biology of Reproduction*, *40*(183-197). https ://doi.org/10.1095/biolreprod41.1.183

Aitken, R. J. (1990). Development of in vitro tests of human sperm function: A diagnostic tool and model system for toxicological analyses. *Toxicology in Vitro*, *4*(4–5), 560–569. https://doi.org/10.1016/0887-2333(90)90116-B

Aitken, R. J., Buckingham, D., & Harkiss, D. (1993). Use of a xanthine oxidase free radical generating system to investigate the cytotoxic effects of reactive oxygen species on human spermatozoa. *Journal of Reproduction and Fertility*. https://doi.org/10.1530/jrf.0.0970441

Aitken, J., & Fisher, H. (1994). Reactive oxygen species generation and human spermatozoa: The balance of benefit and risk. In *BioEssays*. https://doi.org/10.1002/bies.950160409

Aitken, R. J. (1995). Free radicals, lipid peroxidation and sperm function. *Reproduction, Fertility and Development*, *7*(4), 659. https://doi.org/10.1071/RD9950659

Aitken, R. J., & Krausz, C. (2001). Oxidative stress, DNA damage and the Y chromosome. *Reproduction*, *122*(4), 497–506. https://doi.org/10.1530/rep.0.1220497

Aitken, R. J, & Baker, M. A. (2008). The role of proteomics in understanding sperm cell biology. In *International Journal of Andrology*. https://doi.org/10.1111/j.1365-2605.2007.00851.x

Aitken, R. J, Gibb, Z., Mitchell, L. A., Lambourne, S. R., Connaughton, H. S., & De Iuliis, G.

N. (2012). Sperm motility is lost in vitro as a consequence of mitochondrial free radical production and the generation of electrophilic aldehydes but can be significantly rescued by the presence of nucleophilic thiols. *Biology of Reproduction*, *87*(5). https://doi.org/10.1095/biolreprod.112.102020

Alabi, N. S., Whanger, P. D., & Wu, A. S. H. (1985). Interactive effects of organic and inorganic selenium with cadmium and mercury on spermatozoal oxygen consumption and motility in vitro. *Biology of Reproduction*, 33(4), 911–919. https://doi.org/10.1095/biolreprod33.4.911

Alkmim, J.F. Ocorrência de arsênio, cádmio e chumbo em tecidos de aves, suínos, bovinos de corte e equinos no Brasil. 2011. 146f. Tese. (Doutorado em Ciência Animal) - Universidade Federal de Minas Gerais, Belo Horizonte, MG, 2011

Alvarez, J. G., & Storey, B. T. (1984). Assessment of cell damage caused by spontaneous lipid peroxidation in rabbit spermatozoa. *Biology of Reproduction*, *30*(2), 323–331. https://doi.org/10.1095/biolreprod30.2.323

Amidi, F., Pazhohan, A., Shabani Nashtaei, M., Khodarahmian, M., & Nekoonam, S. (2016). The role of antioxidants in sperm freezing: a review. In *Cell and Tissue Banking*. https://doi.org/10.1007/s10561-016-9566-5

Anahara, R., Toyama, Y., Maekawa, M., Kai, M., Ishino, F., Toshimori, K., & Mori, C. (2006). Flutamide depresses expression of cortactin in the ectoplasmic specialization between the Sertoli cells and spermatids in the mouse testis. *Food and Chemical Toxicology*, *44*(7), 1050–1056. https://doi.org/10.1016/j.fct.2005.12.010

Anchordoquy, J. P., Anchordoquy, J. M., Lizarraga, R. M., Nikoloff, N., Pascua, A. M., & Furnus, C. C. (2019). The importance of trace minerals copper, manganese, selenium and zinc in bovine sperm-zona pellucida binding. *Zygote*, *27*(2), 89–96. https://doi.org/10.1017/S0967199419000078

Anirudhan, T. S., & Sreekumari, S. S. (2011). Adsorptive removal of heavy metal ions from industrial effluents using activated carbon derived from waste coconut buttons. *Journal of Environmental Sciences*, *23*(12), 1989–1998. https://doi.org/10.1016/S1001-0742(10)60515-3

Ansa, A. A., Akpere, O., & Imasuen, J. A. (2017). Semen traits, testicular morphometry and histopathology of cadmium-exposed rabbit bucks administered methanolic extract of *Phoenix dactylifera* fruit. *Acta Scientiarum. Animal Sciences*, *39*(2), 207 https://doi.org/10.4025/actascianimsci.v39i2.32858

Appenroth, K. J. (2010). Soil Heavy Metals. *Crossroads*, *19*(Im), 1–18. https://doi.org/10.1007/978-3-642-02436-8

Arabi, M. (2005). Bull spermatozoa under mercury stress. *Reproduction in Domestic Animals*, *40*(5), 454–459. https://doi.org/10.1111/j.1439-0531.2005.00607.x

Arabi, M. (2006). The role of mercury in the etiology of sperm dysfunction in holstein bulls. *Asian-Australasian Journal of Animal Sciences, 19,* 335-340. https://doi.org/10.5713/ajas.2006.335

Arangasamy, A., Mayasula, V. K., Manohar, N., Selvaraju, S., Guvvala, P. R., Soren, N. M., Reddy, I. J., Roy, K. S., & Ravindra, J. P. (2018a). Advancement of puberty and enhancement of seminal characteristics by supplementation of trace minerals to bucks. *Theriogenology*, *110*(February), 182–191. https://doi.org/10.1016/j.theriogenology.2018.01.008

Arangasamy, A., Mayasula, V. K., Manohar, N., Selvaraju, S., Rani, G. P., Soren, N. M., Reddy, I. J., & Ravindra, J. P. (2018b). Cryoprotective role of organic Zn and Cu supplementation in goats (Capra hircus) diet. *Cryobiology*, *81*(January), 117–124. https://doi.org/10.1016/j.cryobiol.2018.02.001

Arangasamy, A., Sharma, R. B., Hemalatha, K., Mayasula, V. K., Selvaraju, S., Pushpa Rani, G., Binsila, B. K., Soren, N. M., Reddy, I. J., Ravindra, J. P., & Bhatta, R. (2018c). Relationship of organic mineral supplementation and spermatozoa/white blood cells mRNA in goats. *Animal Reproduction Science*, *197*, 296–304. https://doi.org/10.1016/j.anireprosci.2018.08.044

Ariza, M. E., & Williams, M. V. (1996). Mutagenesis of AS52 cells by low concentrations of lead(II) and Mercury(II). *Environmental and Molecular Mutagenesis*, *27*(1), 30–33. https://doi.org/10.1002/(SICI)1098-2280(1996)27:1<30::AID-EM4>3.0.CO;2-I

Au, D. W. T., Chiang, M. W. L., & Wu, R. S. S. (2000). Effects of cadmium and phenol on motility and ultrastructure of sea urchin and mussel spermatozoa. *Archives of Environmental Contamination and Toxicology*, *38*(4), 455–463. https://doi.org/10.1007/s002449910060

Aurich, C., Ortega, C. F, Peña, F. J., V., Schrammel, N., Morcuende, D., & Aurich, J. (2018). Seasonal changes in the sperm fatty acid composition of Shetland pony stallions. *Theriogenology*, *107*, 149–153 https://doi.org/10.1016/j.theriogenology.2017.11.004

Avila, R., Cruz-González, I., & Tapia, M. (2003). Workshop summary. *Revista Mexicana de Astronomia y Astrofisica: Serie de Conferencias*, *19*(7), 121–124. https://doi.org/10.1063/1.39765

Awal, M., Alam, J., Hossain, M., & Matsumoto, M. (2015). Effect of Arsenic (As) on the Spermatogenesis of Black Bengal Goat (Capra hircus) Reared at the Arsenic Prone Area of Mymensingh District in Bangladesh. *British Biotechnology Journal*. https://doi.org/10.9734/bbj/2015/12325

Baker, M. A., & Aitken, R. J. (2005). Reactive oxygen species in spermatozoa: Methods for monitoring and significance for the origins of genetic disease and infertility. In *Reproductive Biology and Endocrinology*, *3*(1), 67. https://doi.org/10.1186/1477-7827-3-67

Bansal, A. K., & Bilaspuri, G. S. (2008). Effect of ferrous sulphate and ascorbic acid on motility, viability and lipid peroxidation of crossbred cattle bull spermatozoa. *Animal*, *2*(01). https://doi.org/10.1017/S1751731107000961

Bernstam, L., & Nriagu, J. (2000). Molecular aspects of arsenic stress. *Journal of Toxicology and Environmental Health - Part B: Critical Reviews*, *3*(4), 293–322 https://doi.org/10.1080/109374000436355

Binelli, A., Cogni, D., Parolini, M., Riva, C., & Provini, A. (2009a). Cytotoxic and genotoxic effects of in vitro exposure to Triclosan and Trimethoprim on zebra mussel (Dreissena polymorpha) hemocytes. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, *150*(1), 50–56. https://doi.org/10.1016/j.cbpc.2009.02.005

Binelli, A., Cogni, D., Parolini, M., Riva, C., & Provini, A. (2009b). In vivo experiments for the evaluation of genotoxic and cytotoxic effects of Triclosan in Zebra mussel hemocytes. *Aquatic Toxicology*, *91*(3), 238–244. https://doi.org/10.1016/j.aquatox.2008.11.008

Bucak, M. N., Sariözkan, S., Tuncer, P. B., Ulutaş, P. A., & Akçadağ, H. I. (2009). Effect of antioxidants on microscopic semen parameters, lipid peroxidation and antioxidant activities in Angora goat semen following cryopreservation. *Small Ruminant Research*, *81*(2–3), 90–95. https://doi.org/10.1016/j.smallrumres.2008.11.011

Cerolini, S., Maldjian, A., Surai, P., & Noble, R. (2000). Viability, susceptibility to peroxidation and fatty acid composition of boar semen during liquid storage. *Animal Reproduction Science*, *58*(1–2), 99–111. https://doi.org/10.1016/S0378-4320(99)00035-4

Chen, C. S., Chao, H. T., Pan, R. L., & Wei, Y. H. (1997). Hydroxyl radical-induced decline in motility and increase in lipid peroxidation and DNA modification in human sperm. *Biochemistry and Molecular Biology International*, *43*(2), 291–303. https://doi.org/10.1080/15216549700204071

Chen, H., Liu, J., Luo, L., Baig, M. U., Kim, J. M., & Zirkin, B. R. (2005). Vitamin E, aging and Leydig cell steroidogenesis. *Experimental Gerontology*, *40*(8-9), 728–736 https://doi.org/10.1016/j.exger.2005.06.004

Chowdhury, A. (2009). Recent Advances in Heavy Metals Induced Effect on Male Reproductive Function—A Retrospective. *Al Ameen Journal of Medical Sciences, 2(2)Special:37-42*

Chrestensen, C. A., Starke, D. W., & Mieyal, J. J. (2000). Acute cadmium exposure inactivates thioltransferase (Glutaredoxin), inhibits intracellular reduction of protein-glutathionyl-mixed disulfides, and initiates apoptosis. *Journal of Biological Chemistry*, *275*(34), 26556–26565. https://doi.org/10.1074/jbc.M004097200

Cohen, J. (1992). A power primer. *Psychological Bulletin*. https://doi.org/10.1037/0033-2909.112.1.155

Costa, A. G., Borges, A. M., & Soto-Blanco, B. (2020). Metais tóxicos e seus efeitos sobre a reprodução dos animais. Revisão. *Revista Brasileira de Higiene e Sanidade Animal*, *14*(1), 108–124.

Couto-Santos, F., Souza, A. C. F., Bastos, D. S. S., Ervilha, L. O. G., Dias, F. C. R., Araújo, L. de S., Guimarães, S. E. F., Oliveira, L. L. de, & Machado-Neves, M. (2020). Prepubertal exposure to arsenic alters male reproductive parameters in pubertal and adult rats. *Toxicology and Applied Pharmacology*, *409*, 115304. https://doi.org/10.1016/j.taap.2020.115304

Cupertino, M. C., Novaes, R. D., Santos, E. C., Bastos, D. S. S., Marques dos Santos, D. C., do Carmo Queiroz Fialho, M., & Matta, S. L. P. (2017a). Cadmium-induced testicular damage is associated with mineral imbalance, increased antioxidant enzymes activity and protein oxidation in rats. *Life Sciences*, *175*, 23–30. https://doi.org/10.1016/j.lfs.2017.03.007

Cupertino, M. C., Novaes, R. D., Santos, E. C., Neves, A. C., Silva, E., Oliveira, J. A., & Matta, S. L. P. (2017b). Differential Susceptibility of Germ and Leydig Cells to Cadmium-Mediated Toxicity: Impact on Testis Structure, Adiponectin Levels, and Steroidogenesis. *Oxidative Medicine and Cellular Longevity*, *2017*. https://doi.org/10.1155/2017/3405089

Da Silva, H. A., Koehler, H. S., De Moraes, A., Guimarães, V. D. A., Hack, E., & Carvalho, P. C. D. F. (2008). Análise da viabilidade econômica da produção de leite a pasto e com suplementos na região dos Campos Gerais - Paraná. *Ciencia Rural*. https://doi.org/10.1590/S0103-84782008000200024

Da Silva, E. F. de S. J. da, Missio, D., Martinez, C. S., Vassallo, D. V., Peçanha, F. M., Leivas, F. G., Brum, D. dos S., & Wiggers, G. A. (2019). Mercury at environmental relevant levels affects spermatozoa function and fertility capacity in bovine sperm. *Journal of Toxicology and Environmental Health - Part A: Current Issues*, *82*(4), 268–278. https://doi.org/10.1080/15287394.2019.1589608

Da Silva, J., Gonçalves, R. V., de Melo, F. C. S. A., Sarandy, M. M., & da Matta, S. L. P. (2020). Cadmium Exposure and Testis Susceptibility: a Systematic Review in Murine Models. *Biological Trace Element Research*. https://doi.org/10.1007/s12011-020-02389-0

Danadevi, K., Rozati, R., Reddy, P. P., & Grover, P. (2003). Semen quality of Indian welders occupationally exposed to nickel and chromium. *Reproductive Toxicology*, *17*(4), 451–456.

https://doi.org/10.1016/S0890-6238(03)00040-6

Davila, R. B., Fontes, M. P. F., Pacheco, A. A., & Ferreira, M. da S. (2020). Heavy metals in iron ore tailings and floodplain soils affected by the Samarco dam collapse in Brazil. *Science of the Total Environment*, *709*, 136151. https://doi.org/10.1016/j.scitotenv.2019.136151

Dawson, E. B., Ritter, S., Harris, W. A., Evans, D. R., & Powell, L. C. (1998). Comparison of sperm viability with seminal plasma metal levels. *Biological Trace Element Research*, *64*(1–3), 215–219. https://doi.org/10.1007/BF02783337

De Angelis, C., Galdiero, M., Pivonello, C., Salzano, C., Gianfrilli, D., Piscitelli, P., Lenzi, A., Colao, A., & Pivonello, R. (2017). The environment and male reproduction: The effect of cadmium exposure on reproductive functions and its implication in fertility. *Reproductive Toxicology*, *73*, 105–127. https://doi.org/10.1016/j.reprotox.2017.07.021

De Flora, S., Bennicelli, C., & Bagnasco, M. (1994). Genotoxicity of mercury compounds. A review. *Mutation Research/Reviews in Genetic Toxicology*, *317*(1), 57–79. https://doi.org/10.1016/0165-1110(94)90012-4

De Lamirande, E., Gagnon, C. (1992). Reactive oxygen species and human spermatozoa. I. Effects on the motility of intact spermatozoa and on sperm axonemes. *Journal of Androl, 13*(5):368–378. https://doi.org/10.1002/j.1939-4640.1992.tb03327.x

De Vizcaya-Ruiz, A., Barbier, O., Ruiz-Ramos, R., & Cebrian, M. E. (2009). Biomarkers of oxidative stress and damage in human populations exposed to arsenic. In *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *674*(1-2), 85–92 https://doi.org/10.1016/j.mrgentox.2008.09.020

Duru, N. K., Morshedi, M., & Oehninger, S. (2000). Effects of hydrogen peroxide on DNA and plasma membrane integrity of human spermatozoa. *Fertility and Sterility*, *74*(6), 1200–1207 https://doi.org/10.1016/S0015-0282(00)01591-0

Dutta, S., Majzoub, A., & Agarwal, A. (2019). Oxidative stress and sperm function: A systematic review on evaluation and management. In *Arab Journal of Urology*, *17*(2), 87–97. https://doi.org/10.1080/2090598X.2019.1599624

El Sisy, G. A., Abo El-Maaty, A. M., & Rawash, Z. M. (2016). Comparative blood and seminal plasma oxidant/antioxidant status of Arab stallions with different ages and their relation to semen quality. *Asian Pacific Journal of Reproduction*, *5*(5), 428–433. https://doi.org/10.1016/j.apjr.2016.07.006

Engwa, G. A, Ferdinand, P. U, Nwalo, F. N, & Unachukwu, M. N. (2019). Mechanism and Health Effects of Heavy Metal Toxicity in Humans. In *Poisoning in the Modern World - New*

Tricks for an Old Dog? https://doi.org/10.5772/intechopen.82511

Eskandari, F., & Momeni, H. R. (2016a). Protective effect of silymarin on viability, motility and mitochondrial membrane potential of ram sperm treated with sodium arsenite. *International Journal of Reproductive BioMedicine*, *14*(6), 397–402. https://doi.org/10.29252/ijrm.14.6.397

Eskandari, F., & Momeni, H. R. (2016b). Silymarin protects plasma membrane and acrosome integrity in sperm treated with sodium arsenite. *International Journal of Reproductive BioMedicine*, *14*(1), 47–52. https://doi.org/10.29252/ijrm.14.1.47

Fakhri, Y., Saha, N., Miri, A., Baghaei, M., Roomiani, L., Ghaderpoori, M., Taghavi, M., Keramati, H., Bahmani, Z., Moradi, B., Bay, A., & Pouya, R. H. (2018). Metal concentrations in fillet and gill of parrotfish (Scarus ghobban) from the Persian Gulf and implications for human health. *Food and Chemical Toxicology*, *118* https://doi.org/10.1016/j.fct.2018.05.041

Figueiredo-Pereira, M. E., Yakushin, S., & Cohen, G. (1998). Disruption of the intracellular sulfhydryl homeostasis by cadmium- induced oxidative stress leads to protein thiolation and ubiquitination in neuronal cells. *Journal of Biological Chemistry*, *273*(21), 12703–12709. https://doi.org/10.1074/jbc.273.21.12703

Flora, S. J. S., Pachauri, V., & Saxena, G. (2011). Arsenic, Cadmium and Lead. In *Reproductive and Developmental Toxicology*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-382032-7.10033-5

Gibb, Z., Lambourne, S. R., Curry, B. J., Hall, S. E., & Aitken, R. J. (2016). Aldehyde dehydrogenase plays a pivotal role in the maintenance of stallion sperm motility. *Biology of Reproduction*, *94*(6). https://doi.org/10.1095/biolreprod.116.140509

Girotto, L., Espíndola, E. L. G., Gebara, R. C., & Freitas, J. S. (2020). Acute and chronic effects on eadpoles (Lithobates catesbeianus) Exposed to mining tailings from the dam rupture in Mariana, MG (Brazil). *Water, Air, and Soil Pollution*, *231*(7). https://doi.org/10.1007/s11270-020-04691-y

Guzick, D. S., Overstreet, J. W., Factor-Litvak, P., Brazil, C. K., Nakajima, S. T., Coutifaris, C., Carson, S. A., Cisneros, P., Steinkampf, M. P., Hill, J. A., Xu, D., & Vogel, D. L. (2001). Sperm Morphology, Motility, and Concentration in Fertile and Infertile Men. *New England Journal of Medicine*, *345*(19), 1388–1393. https://doi.org/10.1056/nejmoa003005

Han, Y., Liang, C., Manthari, R. K., Yu, Y., Gao, Y., Liu, Y., Jiang, S., Tikka, C., Wang, J., & Zhang, J. (2020). Arsenic influences spermatogenesis by disorganizing the elongation of spermatids in adult male mice. *Chemosphere*, *238*, 124650 https://doi.org/10.1016/j.chemosphere.2019.124650

Hansen, J. M., Zhang, H., & Jones, D. P. (2006). Differential oxidation of thioredoxin-1, thioredoxin-2, and glutathione by metal ions. *Free Radical Biology and Medicine*. https://doi.org/10.1016/j.freeradbiomed.2005.09.023

Hardneck, F., Israel, G., Pool, E., & Maree, L. (2018). Quantitative assessment of heavy metal effects on sperm function using computer-aided sperm analysis and cytotoxicity assays. *Andrologia*, *50*(10), 1–9. https://doi.org/10.1111/and.13141

Henry, M., Ferreira Brito, M., Neves, B. P., Auler, P. A., Almeida, J., Andrade, G. O., Becerra, V. B., & Bergmann, L. (2017). Peculiarities of the buffalo species for andrological evaluation – results of four years of study and weekly semen collection schedule. *Animal Reproduction*, *14*, (Suppl.1), 1225-1233 https://doi.org/10.21451/1984-3143-AR0005

Hernández-Meléndez, J., Salem, A. Z. M., Sánchez-Dávila, F., Rojo, R., Limas, A. G., López-Aguirre, D., & Vázquez-Armijo, J. F. (2015). Effect of copper and zinc supplementation on growth, blood serum copper and zinc levels, scrotal circumference and semen quality in growing male boer x nubian bucks. *Life Science Journal*, *12*(2), 108–112. https://doi.org/10.7537/marslsj1202s15.17

Herschy, R. W. (2012). Water quality for drinking: WHO guidelines. *Encyclopedia of Earth Sciences Series*, 876–883. https://doi.org/10.1007/978-1-4020-4410-6_184

Hogue, D. E., Parrish, J. J., Foote, R. H., & Stouffer, J. R. (1984). Toxicologic studies with male sheep grazing on municipal sludge-amended soil. *Journal of Toxicology and Environmental Health*, *14*(2-3), 153–161. https://doi.org/10.1080/15287398409530570

Hornsby, P. J. (1989). Steroid and xenobiotic effects on the adrenal cortex: mediation by oxidative and other mechanisms. In *Free Radical Biology and Medicine*, *6*(1), 103–115. https://doi.org/10.1016/0891-5849(89)90163-9

Hosseini, M. J., Shaki, F., Ghazi-Khansari, M., & Pourahmad, J. (2013). Toxicity of arsenic (III) on isolated liver mitochondria: A new mechanistic approach. *Iranian Journal of Pharmaceutical Research*. https://doi.org/10.22037/ijpr.2013.1279

Jiménez-Rabadán, P., Soler, A.J., Ramón, M., García-Álvarez, O., Maroto-Morales, A., Iniesta-Cuerda, M., Fernández-Santos, M.R., Montoro, V., Pérez-Guzmán, M.D., Garde, J.J. (2016). Influence of semen collection method on sperm cryoresistance in small ruminants. *Animal Reproduction Science*, 167, 103–108. https://doi.org/10.1016/j.anireprosci.2016.02.013

Jones, R., Mann, T., & Sherins, R. (1979). Peroxidative breakdown of phospholipids in human spermatozoa, spermicidal properties of fatty acid peroxides, and protective action of seminal plasma. *Fertility and Sterility*. https://doi.org/10.1016/S0015-0282(16)43999-3

Kalender, S., Uzun, F. G., Demir, F., Uzunhisarcikli, M., & Aslanturk, A. (2013). Mercuric chloride-induced testicular toxicity in rats and the protective role of sodium selenite and vitamin E. *Food and Chemical Toxicology*, *55*, 456–462 https://doi.org/10.1016/j.fct.2013.01.024

Kamel, F., & Kubajak, C. L. (1987). Modulation of gonadotropin secretion by corticosterone: Interaction with gonadal steroids and mechanism of action. *Endocrinology*, *121*(2), 561–568. https://doi.org/10.1210/endo-121-2-561

Kasprzak, K. S. (1995). Possible role of oxidative damage in metal-induced carcinogenesis. *Cancer Investigation*, *13*(4), 411–430. https://doi.org/10.3109/07357909509031921

Kendall, N. R., Jackson, D. W., Mackenzie, A. M., Illingworth, D. V., Gill, I. M., & Telfer, S. B. (2001). The effect of a zinc, cobalt and selenium soluble glass bolus on the trace element status of extensively grazed sheep over winter. *Animal Science*, *73*(1), 163–169. https://doi.org/10.1017/S135772980005815X

Kerkeni, L., Ruano, P., Delgado, L. L., Picco, S., Villegas, L., Tonelli, F., Merlo, M., Rigau, J., Diaz, D., & Masuelli, M. (2016). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. *Intech*, *tourism*, 13. https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics

Knazicka, Z., Tvrdá, E., Bardos, L., & Lukac, N. (2012). Dose- and time-dependent effect of copper ions on the viability of bull spermatozoa in different media. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering*, *47*(9), 1294–1300. https://doi.org/10.1080/10934529.2012.672135

Koizumi, T., & Li, Z. G. (1992). Role of oxidative stress in single-dose, cadmium-induced testicular cancer. *Journal of Toxicology and Environmental Health*, *37*(1), 25–36. https://doi.org/10.1080/15287399209531654

Kumar, M., Gogoi, A., Kumari, D., Borah, R., Das, P., Mazumder, P., & Tyagi, V. K. (2017). Review of Perspective, Problems, Challenges, and Future Scenario of Metal Contamination in the Urban Environment. *Journal of Hazardous, Toxic, and Radioactive Waste*, *21*(4). https://doi.org/10.1061/(asce)hz.2153-5515.0000351

Kushawaha, B., Yadav, R. S., Swain, D. K., Rai, P. K., & Garg, S. K. (2020). Mercury-Induced Inhibition of Tyrosine Phosphorylation of Sperm Proteins and Altered Functional Dynamics of Buck Spermatozoa: an In Vitro Study. *Biological Trace Element Research*, *198*(2), 478–492. https://doi.org/10.1007/s12011-020-02077-z

Lapointe, S., Ahmad, I., Buhr, M. M., & Sirard, M. A. (1996). Modulation of postthaw motility, survival, calcium uptake, and fertility of bovine sperm by magnesium and manganese. *Journal of Dairy Science*. https://doi.org/10.3168/jds.S0022-0302(96)76592-X

Leahy, T., Rickard, J. P., Aitken, R. J., & de Graaf, S. P. (2016). D-penicillamine prevents ram sperm agglutination by reducing the disulphide bonds of a copper-binding sperm protein. *Reproduction*, 151(5), 491–500. https://doi.org/10.1530/rep-15-0596

Leita, L., Enne, G., De Nobili, M., Baldini, M., & Sequi, P. (1991). Heavy metal bioaccumulation in lamb and sheep bred in smelting and mining areas of S.W. sardinia (Italy). *Bulletin of Environmental Contamination and Toxicology*, 46(6), 887–893. https://doi.org/10.1007/BF01689734

Li, S. G., Ding, Y. S., Niu, Q., Xu, S. Z., Pang, L. J., Ma, R. L., Jing, M. X., Feng, G. L., Liu, J. M., & Guo, S. X. (2015). Grape seed proanthocyanidin extract alleviates arsenic-induced oxidative reproductive toxicity in male mice. *Biomedical and Environmental Sciences*, *28*(4), 272-280. https://doi.org/10.3967/bes2015.038

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P. A., Clarke, M., Devereaux, P. J., Kleijnen, J., & Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Journal of Clinical Epidemiology*, *62*(10), e1–e34. https://doi.org/10.1016/j.jclinepi.2009.06.006

Lymberopoulos, A. G., Kotsaki-Kovatsi, V. P., Taylor, A., Papaioannou, N., & Brikas, P. (2000). Effects of cadmium chloride administration on the macroscopic and microscopic characteristics of ejaculates from chios ram-lambs. *Theriogenology*. https://doi.org/10.1016/S0093-691X(00)00422-2

Lymberopoulos, A. G., Kotsaki-Kovatsi, V. P., Papaioannou, N., Taylor, A., Brikas, P., & Belibasaki, S. (2003). Effects of cadmium chloride administration on the testicular growth and plasma testosterone secretion of Chios ram-lambs. *Small Ruminant Research*, *49*(1), 51–60. https://doi.org/10.1016/S0921-4488(03)00061-0

Maiga, A., Diallo, D., Bye, R., & Paulsen, B. S. (2005). Determination of some toxic and essential metal ions in medicinal and edible plants from Mali. *Journal of Agricultural and Food Chemistry*, *53*(6), 2316–2321. https://doi.org/10.1021/jf040436o

Mann T. (1964). Male accessory organs of reproduction, and their secretory product: the seminal plasma. In: Mann T. (Ed.) The Biochemistry of Semen and of the Male Reproductive Tract. London, U.K., Methuen,. Pp. 37-78.

Mao, T., Han, C., Wei, B., Zhao, L., Zhang, Q., Deng, R., Liu, J., Luo, Y., & Zhang, Y. (2018). Protective Effects of quercetin against cadmium chloride-induced oxidative injury in goat sperm and zygotes. *Biological Trace Element Research*. https://doi.org/10.1007/s12011-018-1255-8

Martinez, C. S., Peçanha, F. M., Brum, D. S., Santos, F. W., Franco, J. L., Zemolin, A. P. P.,

Anselmo-Franci, J. A., Junior, F. B., Alonso, M. J., Salaices, M., Vassallo, D. V., Leivas, F. G., & Wiggers, G. A. (2017). Reproductive dysfunction after mercury exposure at low levels: Evidence for a role of glutathione peroxidase (GPx) 1 and GPx4 in male rats. *Reproduction, Fertility and Development*, *29*(9), 1803–1812. https://doi.org/10.1071/RD16310

Martínez, J., Limas, T., & Perón, N. (1994). Daily production and testicular and epididymal sperm reserves of Pelibuey rams. *Theriogenology*. https://doi.org/10.1016/0093-691X(94)90824-3

Massányi, P., Lukáč, N., & Trandžík, J. (1996). In vitro inhibition of the motility of bovine spermatozoa by cadmium chloride. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering*, *31*(8), 1865–1879. https://doi.org/10.1080/10934529609376462

Mayasula, V. K., Arangasamy, A., Selvaraju, S., Guvvala, P. R., & Ramesh, K. (2019). Organic Zn and Cu interaction impact on sexual behaviour, semen characteristics, hormones and spermatozoal gene expression in bucks (Capra hircus). *Theriogenology*, *130*, 130–139. https://doi.org/10.1016/j.theriogenology.2019.02.026

Mayasula, V. K., Arunachalam, A., Sellappan, S., Guvvala, P. R., Naidu, S. J., Dintaran, P., & Bhatta, R. (2020). Organic Zn and Cu supplementation imprints on seminal plasma mineral, biochemical/ antioxidant activities and its relationship to spermatozoal characteristics in bucks. *Reproductive Biology*, *20*(2), 220–228. https://doi.org/10.1016/j.repbio.2020.02.007

Mendiola, J., Moreno, J. M., Roca, M., Vergara-Jurez, N., Martínez-García, M. J., García-Snchez, A., Elvira-Rendueles, B., Moreno-Grau, S., Lápez-Espín, J. J., Ten, J., Bernabeu, R., & Torres-Cantero, A. M. (2011). Relationships between heavy metal concentrations in three different body fluids and male reproductive parameters: A pilot study. *Environmental Health: A Global Access Science Source*. https://doi.org/10.1186/1476-069X-10-6

Mertz, W. (1981). The essential trace elements. *Science*, *213*(4514), 1332–1338 https://doi.org/10.1126/science.7022654

Mohammadi, Z., Tavalaee, M., Gharagozloo, P., Drevet, J. R., & Nasr-Esfahani, M. H. (2020). Could high DNA stainability (HDS) be a valuable indicator of sperm nuclear integrity? *Basic and Clinical Andrology*, *30*(12). https://doi.org/10.1186/s12610-020-00110-8

Mouro, V. G. S., Siman, V. A., da Silva, J., Dias, F. C. R., Damasceno, E. M., Cupertino, M. do C., de Melo, F. C. S. A., & da Matta, S. L. P. (2020). Cadmium-Induced Testicular Toxicity in Mice: Subacute and Subchronic Route-Dependent Effects. *Biological Trace Element Research*, *193*(2), 466–482. https://doi.org/10.1007/s12011-019-01731-5

Narasimhaiah, M., Arunachalam, A., Sellappan, S., Mayasula, V. K., Guvvala, P. R., Ghosh, S. K., Chandra, V., Ghosh, J., & Kumar, H. (2018). Organic zinc and copper supplementation on

antioxidant protective mechanism and their correlation with sperm functional characteristics in goats. *Reproduction in Domestic Animals*, *53*(3), 644–654. https://doi.org/10.1111/rda.13154

Peltola, V., Huhtaniemi, I., Metsa-Ketela, T., & Ahotupa, M. (1996). Induction of lipid peroxidation during steroidogenesis in the rat testis. *Endocrinology*, *137*(1), 105–112. https://doi.org/10.1210/endo.137.1.8536600

Predes, F. S., Diamante, M. A. S., & Dolder, H. (2010). Testis response to low doses of cadmium in Wistar rats. *International Journal of Experimental Pathology*, *91*(2), 125–131 https://doi.org/10.1111/j.1365-2613.2009.00692.x

Pizent, A., Tariba, B., & Živković, T. (2012). Reproductive toxicity of metals in men. *Archives of Industrial Hygiene and Toxicology*, 63(Supplement-1). https://doi.org/10.2478/10004-1254-63-2012-2151

Qadori, Y. T., & Al-shaikh, M. N. (2012). Effects of high and low dose of cadmium chloride on male Reproductive system in mice. *Journal of the Faculty of Medicine Baghdad, 54*(110), 110–114.

Quadra, G. R., Roland, F., Barros, N., Malm, O., Lino, A. S., Azevedo, G. M., … Campos, J. M. S. (2018). Far-reaching cytogenotoxic effects of mine waste from the Fundão dam disaster in Brazil. *Chemosphere*, *215*, 753–757. https://doi.org/10.1016/j.chemosphere.2018.10.104

Rehman, K., Fatima, F., Waheed, I., & Akash, M. S. H. (2018). Prevalence of exposure of heavy metals and their impact on health consequences. *Journal of Cellular Biochemistry*, *119*(1), 157–184. https://doi.org/10.1002/jcb.26234

Renu, K., Madhyastha, H., Madhyastha, R., Maruyama, M., Vinayagam, S., & Valsala Gopalakrishnan, A. (2018). Review on molecular and biochemical insights of arsenic-mediated male reproductive toxicity. *Life Sciences*, *212*(October), 37–58. https://doi.org/10.1016/j.lfs.2018.09.045

Rosenberg, M. S., Adams, D.C., & Gurevitch, J. (2000). MetaWin: Statistical Software for Meta-Analysis: Version 2.0, Sinauer Associates.

Roy, D., Dey, S., Majumder, G. C., & Bhattacharyya, D. (2014a). Copper: A biphasic regulator of caprine sperm forward progression. *Systems Biology in Reproductive Medicine*, *60*(1), 52–57. https://doi.org/10.3109/19396368.2013.848243

Roy, D., Dey, S., Majumder, G. C., & Bhattacharyya, D. (2014b). Occurrence of novel Cu2+-dependent sialic acid-specific lectin, on the outer surface of mature caprine spermatozoa. *Glycoconjugate Journal*, *31*(4), 281–288. https://doi.org/10.1007/s10719-014-9524-z

Salamon, S., & Maxwell, W. M. (2000). Storage of ram semen. *Animal Reproduction Science*, *62*(1-3), 77–111. https://doi.org/10.1016/S0378-4320(00)00155-X

Sall, M. L., Diaw, A. K. D., Gningue-Sall, D., Efremova Aaron, S., & Aaron, J.-J. (2020). Toxic heavy metals: impact on the environment and human health, and treatment with conducting organic polymers, a review. *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-020-09354-3

Saleh, R. A., Agarwal, A., Nada, E. A., El-Tonsy, M. H., Sharma, R. K., Meyer, A., Nelson, D. R., & Thomas, A. J. (2003). Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. *Fertility and Sterility*, *79*(SUPPL. 3), 1597–1605. https://doi.org/10.1016/S0015-0282(03)00337-6

Sandrini, J. Z., Bianchini, A., Trindade, G. S., Nery, L. E. M., & Marins, L. F. F. (2009). Reactive oxygen species generation and expression of DNA repair-related genes after copper exposure in zebrafish (Danio rerio) ZFL cells. *Aquatic Toxicology*, *95*(4), 285–291. https://doi.org/10.1016/j.aquatox.2009.02.016

Selvaraju, S., Nandi, S., Gupta, P. S. P., & Ravindra, J. P. (2011). Effects of heavy metals and pesticides on buffalo (Bubalus bubalis) spermatozoa functions in vitro. *Reproduction in Domestic Animals*, *46*(5), 807–813. https://doi.org/10.1111/j.1439-0531.2010.01745.x

Shen, Z. Y., Shen, J., Cai, W. J., Hong, C., & Zheng, M. H. (2000). The alteration of mitochondria is an early event of arsenic trioxide induced apoptosis in esophageal carcinoma cells. *International Journal of Molecular Medicine*. https://doi.org/10.3892/ijmm.5.2.155

Shenker, B. J., Guo, T. L., & Shapiro, I. M. (2000). Mercury-induced apoptosis in human lymphoid cells: Evidence that the apoptotic pathway is mercurial species dependent. *Environmental Research*, *84*(2), 89–99. https://doi.org/10.1006/enrs.2000.4078

Silva, M. L. de S., Vitti, G. C., & Trevizam, A. R. (2007). Concentração de metais pesados em grãos de plantas cultivadas em solo com diferentes níveis de contaminação. *Pesquisa Agropecuária Brasileira*, *42*(4), 527–535. https://doi.org/10.1590/s0100-204x2007000400011

Singh, P., Chand, D., & Georgie, G. C. (1989). Effect of vitamin E on lipid peroxidation in Buffalo Bubalus bubalis L. *Indian Journal of Experimental Biology*, *27*(1):14-6

Sousa, F. M. L., Lobo, C. H., Menezes, E. S. B., Rego, J. P. A., Oliveira, R. V., Lima-Souza, A. C., Fioramonte, M., Gozzo, F. C., Pompeu, R. C. F. F., Cândido, M. J. D., Oliveira, J. T., & Moura, A. A. (2014). Parameters of the reproductive tract, spermatogenesis, daily sperm production and major seminal plasma proteins of tropically adapted morada nova rams. *Reproduction in Domestic Animals*. https://doi.org/10.1111/rda.12288

Souza, M. V. de, Vianna, M. W. de S., Zandim, B. M., Fernandes, R. B. A., & Fontes, M. P. F. (2009). Metais pesados em amostras biológicas de bovinos. *Ciência Rural*, 39(6), 1774–1781. https://doi.org/10.1590/S0103-84782009005000088

Souza, A. C. F., Bastos, D. S. S., Santos, F. C., Sertorio, M. N., Ervilha, L. O. G., Gonçalves, R. V., de Oliveira, L. L., Machado-Neves, M. (2018). Arsenic aggravates oxidative stress causing hepatic alterations and inflammation in diabetic rats. *Life Sciences*. doi:10.1016/j.lfs.2018.08.054

Souza, A. C. F., de Paiva Coimbra, J. L., Ervilha, L. O. G., Bastos, D. S. S., Cossolin, J. F. S., Santos, E. C., de Oliveira, L. L., Machado-Neves, M. (2020). Arsenic induces dose-dependent structural and ultrastructural pathological remodeling in the heart of Wistar rats. *Life Sciences*, 118132. https://doi.org/10.1016/j.lfs.2020.118132

Tavares, T. M., Carvalho, F. Avaliação de exposição de populações humanas a metais pesados no ambiente: exemplos do Recôncavo Baiano. (1992). *Quimica Nova*, *15(2):147-154*

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy Metal Toxicity and the Environment. Molecular, Clinical and Environmental Toxicology, 133–164. https://doi.org/10.1007/978-3-7643-8340-4_6

Thompson, F., de Oliveira, B. C., Cordeiro, M. C., Masi, B. P., Rangel, T. P., Paz, P., … de Rezende, C. E. (2019). Severe impacts of the Brumadinho dam failure (Minas Gerais, Brazil) on the water quality of the Paraopeba River. *Science of the Total Environment*, *705*, 135914. https://doi.org/10.1016/j.scitotenv.2019.135914

Tvrdá, E., Lukáč, N., Lukáčová, J., Jambor, T., & Massányi, P. (2015a). Dose- and Time-Dependent In Vitro Effects of Divalent and Trivalent Iron on the Activity of Bovine Spermatozoa. *Biological Trace Element Research*, *167*(1), 36–47. https://doi.org/10.1007/s12011-015-0288-5

Tvrdá, E., Kováčik, A., Tušimová, E., Massányi, P., & Lukáč, N. (2015b). Resveratrol offers protection to oxidative stress induced by ferrous ascorbate in bovine spermatozoa. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering*, *50*(14), 1440–1451. https://doi.org/10.1080/10934529.2015.1071153

Vergilio, C. dos S., Lacerda, D., Oliveira, B. C. V. de, Sartori, E., Campos, G. M., Pereira, A. L. de S., … Rezende, C. E. de. (2020). Metal concentrations and biological effects from one of the largest mining disasters in the world (Brumadinho, Minas Gerais, Brazil). *Scientific Reports*, *10*(1), 1–12. https://doi.org/10.1038/s41598-020-62700-w

Xu, D. X., Shen, H. M., Zhu, Q. X., Chua, L., Wang, Q. N., Chia, S. E., & Ong, C. N. (2003). The associations among semen quality, oxidative DNA damage in human spermatozoa and concentrations of cadmium, lead and selenium in seminal plasma. *Mutation Research - Genetic*

Toxicology and Environmental Mutagenesis, *534*(1–2), 155–163. https://doi.org/10.1016/S1383-5718(02)00274-7

World Health Organization (WHO). 2017. Guidelines for drinking-water quality: fourth edition incorporating the first addendum. Geneva

Yang, J. L., Chao, J. I., & Lin, J. G. (1996). Reactive oxygen species may participate in the mutagenicity and mutational spectrum of cadmium in Chinese Hamster Ovary-K1 cells. *Chemical Research in Toxicology*, *9*(8), 1360–1367. https://doi.org/10.1021/tx960122y

Yousef, M. I., El-Demerdash, F. M., Kamil, K. I., & Elaswad, F. A. M. (2006). Ameliorating effect of folic acid on chromium(VI)-induced changes in reproductive performance and seminal plasma biochemistry in male rabbits. *Reproductive Toxicology*, *21*(3), 322–328. https://doi.org/10.1016/j.reprotox.2005.09.005

Zhang, F., Xu, Z., Gao, J., Xu, B., & Deng, Y. (2008). In vitro effect of manganese chloride exposure on energy metabolism and oxidative damage of mitochondria isolated from rat brain. *Environmental Toxicology and Pharmacology*, *26*(2), 232–236. https://doi.org/10.1016/j.etap.2008.04.003

Zubair, Muhammad, Ahmad, M., Ahmad, N., Naveed, M. R., Idrees, M., Sallam, M. A., & Bashir, M. I. (2014). Toxic effects of arsenic on reproductive functions of male rabbit and their amelioration with vitamin E. *Global Veterinaria*, *12*(2), 213–218. https://doi.org/10.5829/idosi.gv.2014.12.02.82126

Zubair, M., Ahmad, M., Jamil, H., & Deeba, F. (2016a). Toxic effects of arsenic on semen and hormonal profile and their amelioration with vitamin E in Teddy goat bucks. *Andrologia*, *48*(10), 1220–1228. https://doi.org/10.1111/and.12564

Zubair, M., Ahmad, M., Saleemi, M. K., Gul, S. T., Ahmad, N., & Umar, S. (2016b). Protective effects of vitamin E on sodium arsenite-induced toxicity, testicular measurements and histopathological studies of testes in Teddy goat bucks. *Andrologia*, *49*(7), 1–6. https://doi.org/10.1111/and.12699

Zubair, Muhammad, Ahmad, M., Saleemi, M. K., Gul, S. T., Ahmad, M., Martyniuk, C. J., Ullah, Q., & Umar, S. (2020). Sodium arsenite toxicity on hematology indices and reproductive parameters in Teddy goat bucks and their amelioration with vitamin C. *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-020-08049-

Supplementary Table S1. Exclusion reasons for the metals that did not meet the inclusion criteria of the current meta-analysis.

Metal	**Identification** *Records identified through database search*	**Screening:** *Records screened by title and abstract*	**Eligibility:** *Full-text articles assessed for eligibility*	**Included:** *Articles eligible for meta-analyses*
Aluminum	Total number of studies identified = 642 • PubMed/MEDLINE (n = 199) • Web of Science (n = 132) • Scopus (n = 311)	Records excluded (n = 404): • Duplicates (294) • Not aluminum exposure (154) • Not domestic ruminants as animal model (193)	Records excluded (n = 1): • Content not applicable (1)	N studies = 0
Cobalt	Total number of studies identified = 1.025 • PubMed/MEDLINE (n = 337) • Web of Science (n = 229) • Scopus (n = 459)	Records excluded (n = 1.016): • Duplicates (471) • Not cobalt exposure (408) • Not sperm* studies (63) • Not domestic ruminants as animal model (72) • Review studies (2)	Records excluded (n = 9): • Content not applicable (3) • Statistical data not reported (4) • Full-text not available (2) •	N studies = 0
Chromium	Total number of studies identified = 618 • PubMed/MEDLINE (n = 150) • Web of Science (n = 184) • Scopus (n = 284)	Records excluded (n = 617): • Duplicates (341) • Not chromium exposure (117) • Not domestic ruminants as animal models (127)	Records excluded (n = 1): • Not adequate statistical data (1)	N studies = 0

		• Not sperm* studies (27) • Review studies (5)		
Lead	Total number of studies identified = 904 • PubMed/MEDLINE (n = 133) • Web of Science (n = 411) • Scopus (n = 360)	Records excluded (n = 900): • Duplicates (371) • Not lead exposure (320) • Not domestic ruminants as animal models (192) • Not sperm* studies (8) • Review studies (9)	Records excluded (n = 4): • Content not applicable (2) • Statistical data not reported (1) • Full-text not available (1)	N studies = 0
Nickel	Total number of studies identified = 814 • PubMed/MEDLINE (n = 261) • Web of Science (n = 216) • Scopus (n = 337)	Records excluded (n = 802): • Duplicates (399) • Not nickel exposure (237) • Not sperm* studies (61) • Not domestic ruminants as animal model (98) • Review studies (7)	Records excluded (n = 12): • Content not applicable (8) • Statistical data not reported (1) • Not adequate statistical data (2) • Full-text not available (1)	N studies = 0
Magnesium	Total number of studies identified = 1.112 • PubMed/MEDLINE (n = 358) • Web of Science (n = 105) • Scopus (n = 649)	Records excluded (n = 1.103): • Duplicates (530) • Not magnesium exposure (510) • Not sperm* studies (35) • Not domestic ruminants as animal model (28)	Records excluded (n = 9): • Content not applicable (3) • Statistical data not reported (3) • Not adequate statistical data (3)	N studies = 0

N: number of studies

Supplementary Table S2. Detailed qualitative data of each study included in this meta-analysis review.

Metal	**Animal model**	**Chemical element**	**Observational studies**	**Methodological design of *in vitro* studies**	**Methodological design of *in vivo* study**	**Sperm source**	**Semen collection**	**Number of independent comparisons***
Arsenic (4)	Goat (2) Ram (2)	Sodium arsenite (4)	-	(2): up to 1 d incubation at concentrations up to 1.99 mg/L, in single doses	(2): orally on diet at concentrations between 5 to 65 mg/L, in daily doses during 7 to 84 d	Ejaculate (2) Epididymal (2)	AV (2) SW (2)	19
Cadmium (8)	Ram (4) Bull (2) Goat (1) Buffalo (1)	Cadmium chloride (7) Unknown chemical form (1)	(1): orally on diet at concentration of more than 80 mg/L, in daily doses for longer than 100 d	(5): up to 1 d incubation at concentrations up to 1.99, 5 to 65 and more than 80 mg/L, in single doses	(2): orally by dosimetric syringe at concentration between 5 to 65 mg/L, in daily doses during 7 to 84 d and for longer than 100 d	Ejaculate (7) Epididymal (1)	AV (3) EE (4) SW (1)	44

Copper (13)	Goat (8) Bull (3) Ram (1) Buffalo (1)	Organic copper (6) Cu^{2+} (3) Copper sulphate (3) Unknown chemical form (1)	-	(7): up to 1 d incubation at concentrations up to 1.99 and 5 to 65 mg/L, in single doses	(6): orally on diet at concentrations of more than 80 mg/L, in daily doses during 7 to 84 d and for longer than 100 d.	Ejaculate (12) Epididymal (1)	AV (6) EE (6) SW (1)	93
Iron (2)	Bull (2)	Di and trivalent iron chloride (1) Ferrous ascorbate (1)	-	(2): up to 1 d incubation at concentrations between 5 to 65 mg/L, in single doses	-	Ejaculate (2)	AV (2)	11
Manganese (4)	Bull (4)	Manganese chloride (1) Unknown chemical form (1) Divalent manganese (2)	-	(2): up to 1 d incubation at concentrations up to 1.99 and between 5 to 65 mg/L, in single doses	-	Ejaculate (4)	AV (4)	14

Mercury (5)	Bull (3) Goat (1) Ram (1)	Mercury chloride (5)	-	(5): up to 1 d incubation at concentrations up to 1.99, between 5 to 65, and more than 80 mg/L	-	Ejaculate (5)	AV (4) EE (1)	31

d: days; AV: artificial vagina; EE: electro-ejaculator; SW: sperm washing