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REVIEW ARTICLE
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# Systematic Review of Acrylamide-Induced Toxicity and Oxidative Stress in Humans and Animals: Antioxidant Strategies for Male Reproductive Dysfunction

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## ABSTRACT

Food contaminant acrylamide (AA), a byproduct of heat and also a component of tobacco smoke, is metabolized to glycidamide (GA) which have been shown to induce male reproductive toxicity by mechanisms that can largely be described as a consequence of oxidative stress, inflammation, and mitochondrial dysfunctions. As demonstrated in published literature, AA exposure greatly enhances lipid peroxidation and decreases the activity of antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, and glutathione, which cause testicular damage, apoptosis, hormonal imbalance and inhibition of sperm activity. Associations between biomarkers of acrylamide exposure and low semen quality are also reported in public health. Therefore, due to confounding factors, causal interpretation is not possible. The protective effects have indicated that several antioxidants such as vitamins C and E, zinc, selenium, melatonin, N-acetylcysteine, coenzyme Q10 and different polyphenolic compounds on the toxicity of AA-induced testicular and sperm injury through the restoration of redox balance, maintenance of hormonal balance and the enhancement of sperm parameters. Collectively, existing literature supports oxidative stress as a central mechanism in acrylamide-mediated reproductive dysfunctions and highlights antioxidants as potential therapeutic agents, though further controlled clinical studies are required to establish standardized treatment strategies, effective dosages and translational relevance to human male fertility.

**Keywords:** Acrylamide, Glycidamide, Oxidative stress, Male infertility, Testis, Sperm, Antioxidants, Nrf2/Keap1, CYP2E1, DNA damage.

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## INTRODUCTION

Acrylamide (AA) is a small, water-soluble organic molecule formed as a byproduct of high-temperature cooking, particularly in carbohydrate-rich foods such as fried potatoes, baked materials, coffee and toasted cereals. Its formation principally arises through the Maillard reaction between asparagine and reducing sugars at temperatures above about 120°C, making it a common dietary contaminant (Adimas et al., 2024; Rasool et al., 2025). In addition to dietary sources, AA is also present in cigarette smoke and in certain industrial environments, thereby increasing cumulative exposure in humans (Timmermann et al., 2021; Esposito et al., 2022). Once absorbed via the gastrointestinal tract or inhalation, AA is distributed systemically and undergoes metabolic activation, chiefly via cytochrome P450 2E1 (CYP2E1), to its epoxide metabolite glycidamide (GA). GA is considered to be more reactive toward biomolecules than the parent compound. Concurrently, AA and GA can undergo detoxification through conjugation with glutathione (GSH) via glutathione S-transferases (Kocadağlı and Gökmen, 2024; Palus, 2024).

Male reproductive system is prone to oxidative stress. This is due to the fact that sperm cells possess a very small cytoplasm, thus they have a weak capacity to counteract oxidative stress. Meanwhile, they have their outer membranes filled with polyunsaturated fatty acids, which is likely to experience lipid peroxidation (Dutta et al., 2021). Studies on both animals and cells have revealed that exposure to AA and GA can damage the structure of testicles, sperm production and their functionality.

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One of the possible causes of this occurrence appears to be oxidative stress (Alturki et al., 2022; Seify et al., 2024). It is observed that the rodents which were administered AA, contained higher malondialdehyde (MDA) or TBARS in the testicles. However, the antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were not as successful and glutathione (GSH) was in lesser proportions (El-Beltagi et al., 2016; Alturki et al., 2022). These redox disturbances often accompany mitochondrial membrane potential disruption, increases the generation of reactive oxygen species (ROS) and induction of apoptosis—characterized by upregulation of Bax and caspase-3 and downregulation of Bcl-2 in Leydig, Sertoli, or germ cells (Zhang et al., 2023; Seify et al., 2024).

At the histopathological levels, the exposure of animals to AA has caused disruptions in the seminiferous tubules, germ cell loss, vacuolization, interstitial edema and in some cases formation of giant cells and necrosis (Abduljalil et al., 2024; Singh et al., 2024). In addition, steroidogenic imbalance is often reported, with changes in serum testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels, as well as altered expression of key steroidogenic enzymes (Tekin et al., 2025). It is recorded that when exposed to acrylamide (AA), animals and laboratory workers have issues with sperm functionality such as poor movement of sperms, decreased survival rates, deformed shape and broken DNA (Trigg et al., 2021). Exposing human sperm to AA out of the body, researchers have observed the growth of harmful molecules called ROS, the reduction of the sperm fighting the damage, and the damage of the outer part of the sperm (Omidi et al., 2020). Given the formidable evidence and mechanistic possibility of the importance of oxidative stress in AA/GA-induced male reproductive toxicity, a number of antioxidant strategies have been explored in order to suppress its effects. Compounds such as vitamins (E and C), melatonin, zinc, selenium, coenzyme Q10 and polyphenols have been used in different animal and cellular models with encouraging outcomes including restoration of antioxidant enzyme levels; reduction of lipid peroxidation; improvement of apoptosis apoptotic activity; normalization of hormone levels; or recovery of sperm parameters (Shahrzad et al., 2020; Bhuiyan et al., 2023; Ajibare et al., 2024; Elsayed et al., 2024) For example, zinc supplementation significantly decreased testicular MDA and inflammatory markers, but enhanced antioxidant enzyme activities and sperm indices in AA-treated rodents (Ajibare et al., 2024). In addition, Eruca sativa seed extract attenuated ROS in semen and improved DNA integrity following the AA challenge (Abd-Elsalam et al., 2021).

With more and more men facing fertility issues and widespread exposure to acrylamide (AA), it's crucial to bring together the existing research both from laboratories research studies and the small amount from human trials to figure out the best antioxidant treatments. We should understand what works best, how much and over what period are effective and under what circumstances they are productive, in either research or a clinical context. It is through combination of this information that we shall be in a better position to plan future human trials on hard evidence. It will also guide us to make informed diet and treatment recommendations, and know how AA exposure causes issues with male reproductive health on a molecular basis.

## MATERIALS & METHODS

### Study Design and Protocol Registration

This systematic review was planned to critically assess the published information on acrylonitrile (AA) and its metabolite glycidamide (GA)-induced oxidative stress and male reproductive toxicity and the possible protective actions of antioxidant interventions. The review was done in a structured manner and in accordance to the usual systematic review methods to be sure of the transparency of the methods used to ensure methodological reproducibility and exhausting all the available data.

### Research Question and PECO Framework

The research question was formulated based on PECO framework, which stands as Population, Exposure, Comparator, and Outcome, to determine the scope of the reviews. Population consisted of either male animals, human beings or cell-based models wherein male reproductive tissues or sperm cells were used. The exposure was the acrylamide or its metabolite glycidamide at any route, either by mouth, diet, intraperitoneal, or by inhalation. The comparator was control or unexposed groups and the results were biochemical and physiological evidence of oxidative stress like reactive oxygen species (ROS), malondialdehyde (MDA), Thiobarbituric acid-reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH). Other results were hormonal including testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testicular and sperm morphology, apoptosis and histopathological changes. The main aim of the review was to summarize all the pieces of evidence explaining oxidative processes of acrylamide on the male reproductive system and investigate the effectiveness of antioxidant treatments in reducing such toxic effects.

### Search Strategy and Information Sources

An extensive literature review was done in several electronic databases, including PubMed, Scopus, Web of Science, Science Direct and Embase, including articles published between January 2000 and December 2025. Grey literature was captured with the help of supplementary sources like Google Scholar, PakMediNet and by hand screening reference lists of pertinent reviews and primary research articles to ensure completeness. Search strategy used combined Medical Subject Headings (MeSH) and free-text search terms that are related to acryl amide toxicity and antioxidants, that is, the Boolean string: (acryl amide OR glycidamide) AND (male reproduction OR testis OR sperm OR fertility OR Leydig cells OR Sertoli cells) AND (oxidative stress OR ROS OR lipid peroxidation OR MDA OR SOD OR catalase OR glutathione) AND (antioxidant OR vitamin E OR vitamin C.

**Eligibility Criteria**

The inclusion criteria were that the studies were original experimental or observational studies published in peer-reviewed journals and examined the effects of acrylamide or glycidamide exposure on male reproductive or oxidative stress. The included studies had to include quantitative or qualitative data of oxidative biomarkers, hormonal tests, histopathological results or sperm quality parameters and to include a control or comparator group. The articles that were excluded included review articles, editorials, conference abstracts that did not include full data, case reports, studies in non-mammalian species and articles written in other languages other than English.

**Data Extraction and Management**

Extracted information was bibliographic (author, year, country and journal), study design (in vivo, in vitro, clinical or observational) and further experimental details including species or cell line used for the experiments, number of samples included in each experiment set up, strain and age of the animal(s), exposure methods (dose amount given to the animals in drug treatment series; route described presenting how AA has been administered into drug treatment series such as intraperitoneal (i.p.) gavage feedings (g.f.), liquid diet administration (l.d.a.), duration time exposing animals to induce action. Results of oxidative stress markers (SOD, CAT, GPx, GSH, MDA, TBARS and ROS), antioxidant treatments (type of compounds, doses, administrating route and timing) and reproductive results (semen analysis: concentration; motility; morphology; viability tests; testis histopathology and hormone levels) were recorded. Furthermore, significant results and stat data such as the mean±SD, P-values and fold changes were summarized for further cross-analyses.

**Quality Assessment and Risk of Bias**

The quality of each included study was critically appraised with appropriate and validated tools. For animal experiments, potential biases across the domains of selection, performance, detection, attrition and reporting were evaluated using SYRCLE's Risk of Bias (RoB) tool (Hooijmans et al., 2014). Confounding, participant selection, intervention classification and outcome measurement biases in human and observational studies were assessed using the ROBINS-I tool. Each domain of each study was rated as low, unclear or high risk of bias.

**Data Synthesis and Statistical Analysis**

First, we performed a qualitative synthesis to compile results from the experimental models, exposure conditions and antioxidant treatments. Meta-analyses were conducted using Comprehensive Meta-Analysis (CMA) software, version 3.0, when sufficient quantitative evidence (≥ 3 studies with the same outcome measure) was available. Pooled standardized mean difference (SMD) with 95% confidence interval was computed using random-effects model through DerSimonian–Laird method. Statistical heterogeneity between studies was tested by the Q test and $I^2$ statistics, where $I^2$>50% suggested high heterogeneity. Sensitivity analysis was also performed alternatively omitting studies one by one for robustness testing, and subgroup analyzes were carried out to examine the effect of species, treatment dose of exposure, duration, type of antioxidant used and time application (preventive or therapeutic). Publication bias was assessed by visual inspection of funnel plots, and quantitatively examined with Egger's regression test.

**Evidence Certainty and Grading**

The overall quality of evidence for each primary outcome was considered in accordance with GRADE (Grading of Recommendations, Assessment, Development and Evaluation) methodology modified for toxicological studies. Evidence in each domain was evaluated using risk of bias, consistency, directness, and precision and publication bias as criteria. According to this assessment, the certainty of evidence was graded in four levels: high, moderate, low and very low representing certainty of derived conclusions.

**Ethical Considerations**

This was a systematic review and all of the data were extracted from previously published studies; there had been no need for new ethical approval nor informed consent. All original studies included in this review had obtained ethics approval by their institutional or national committees as reported in the publications.

**Acrylamide Exposure and Kinetics**

**Sources and Doses**

Acrylamide is primarily produced in carbohydrate-rich foods like deep-fried potatoes, chips, bread crusts and roasted coffee when they are processed at high temperatures (exceeding 120°C). This occurs by way of a Maillard reaction between the asparagine and some sugars (Friedman, 2015; Aghvami et al., 2023). Studies of processed foods have found acrylamide levels ranging from 200 to 3500 µg/kg in fried or baked foods, with potato chips and French fries often having the highest levels (El-Sheikh et al., 2025). Adults usually consume about 0.2-1.53µg of acrylamide per kilogram of their body weight each day through their diet. Children might consume up to three times as much as adults because they eat more food relative to their body weight (Timmermann et al., 2021). Moreover, Başaran et al. (2023) reported 0.4-0.9µg/kg body weight/day in different populations while EFSA's estimated higher for infant/children group 0.5-1.9µg/kg body weight/day. Additional exposure arises from tobacco smoke, which can contribute approximately 0.5-4.3µg/day, and from occupational environments such as acrylamide polymer manufacturing, tunnel construction, and wastewater treatment plants where dermal and inhalational routes dominate (Kenwood et al., 2022; Louro et al., 2022; Ahmadi et al., 2022; Palus, 2024).

**Absorption, Distribution, Metabolism and Excretion (ADME)**

Acrylamide is rapidly absorbed through both the gastrointestinal and respiratory tracts and readily

distributed throughout the body, including reproductive organs such as the testes (Pietropaoli et al., 2022; Govindaraju et al., 2024). In the liver, it undergoes biotransformation by cytochrome P450 2E1 (CYP2E1) to form glycidamide (GA) a highly reactive epoxide metabolite responsible for much of AA's genotoxic and mutagenic potential (Mori et al., 2022; Ligina et al., 2022; Milanović et al., 2023). A concurrent detoxification pathway involves conjugation of acrylamide and glycidamide with glutathione (GSH) via glutathione S-transferase, producing mercapturic acids that are excreted in urine (Homayoonfal et al., 2024). Our bodies process glycidamide through two competing processes: oxidation by CYP2E1 and GSH conjugation. If CYP2E1 functions more intensively, glycidamide is produced more and has the potential to induce an oxidative insult (Zhang et al., 2023; Marković et al., 2022). Both acrylamide (AA) and glycidamide (GA) can enter the testicles, accumulate in sperm cells and may induce the creation of toxic molecules known as reactive oxygen species (ROS). Such a situation may promote lipid peroxidation and mitochondrial dysfunction, which ultimately interfere with the process of spermatogenesis (Seify et al., 2024; El-Sheikh et al., 2025).

**Biomarkers of Internal Exposure**

Quantification of acrylamide exposure in humans and experimental models relies on two primary biomarker categories: hemoglobin adducts and urinary metabolites. The N-terminal valine adducts of hemoglobin, specifically N-(2-carbamoylethyl) valine (HbAA) and N-(2-carbamoyl-2-hydroxyethyl) valine (HbGA), are the most reliable long-term exposure markers, reflecting cumulative intake over approximately 120 days (Li et al., 2022). Typical background levels in the general population ~ 39pmol/g Hb for HbAA and ~ 34.1pmol/g Hb for HbGA, while smokers and occupationally exposed individuals may exhibit substantially higher adduct concentrations, often reaching hundreds of pmol/g Hb and in some early industrial cohorts exceeding 500pmol/g Hb (Albiach-Delgado et al. 2022; Pedersen et al. 2022; Vryonidis et al. 2024; Poteser et al., 2022; Başaran et al., 2023). Short-term exposure assessment is achieved by detecting urinary mercapturic acids, primarily N-acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA) and N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA), which serve as sensitive indicators of recent intake (Wan et al., 2022; Zhao et al., 2023). These biomarkers have been widely employed to correlate acrylamide exposure with oxidative stress markers and reproductive toxicity in both animal and human studies (Zhang et al., 2023).

Available evidence demonstrates that acrylamide exposure is a ubiquitous risk due to its formation in thermally processed foods and environmental sources. Its efficient absorption, wide distribution to reproductive organs, and conversion to glycidamide through CYP2E1-mediated metabolism underpin its oxidative, genotoxic, and reproductive toxicity, while hemoglobin adducts and urinary mercapturic acids remain the most dependable biomarkers for exposure assessment (Zhang et al., 2025a).

**Mechanisms Linking Acrylamide to Male Reproductive Toxicity**

Acrylamide is able to affect the male reproductive system through numerous mechanisms, mainly by inducing oxidative stress, mitochondrial dysfunction, apoptotic responses, hormone imbalances, DNA damage, inflammation, and altered antioxidant protective mechanisms (Zhang et al., 2023). Oral *In Vivo* and Intraperitoneal *In Vivo* and In Vitro experimental studies on acrylamide toxicity presented in Table 1, 2 and 3 respectively.

**Oxidative Stress as the Central Mechanism**

Acrylamide and its metabolite glycidamide are reported to have significantly increased level of signs for lipid peroxidation, such as TBARS, MDA and simultaneously decreased activity of SOD, CAT, GPx and GSH in the testes (Aydin, 2018; Dos Santos et al., 2023). The increased oxidative stress could lead to the excessive ROS generation and the endoplasmic cell structures damage (Yang et al., 2021). In both rats and mice treated with acrylamide similar results were confirmed, MDA levels increased between two to three-fold, whereas the activity of SOD and CAT diminished in cauda epididymis homogenate samples, which also suggests enhanced lipid peroxidation along with weakened defense mechanism by specific antioxidants such as SOD and CAT (Sedik et al., 2025). Correlation network depicting relationships between oxidative stress and molecular defense markers in Fig. 1. Administration of zinc or vitamin E partly ameliorated antioxidant enzymes' activity, supporting that oxidative imbalance is the key mediator in the testicular toxicity induced by acrylamide (Ajibare et al., 2024; Üremiş et al., 2024a).

**Mitochondrial Dysfunctions**

It has been demonstrated that exposure of cells to AA leads to collapse of the mitochondrial membrane potential ($\Delta\psi m$) and decrease of ATP synthesis, followed by release of apoptogenic cytochrome c into the cytoplasm resulting in activation of intrinsic apoptosis pathway (Pan et al., 2017). Mitochondrial swelling and enhanced LDH activity additionally demonstrate that energy metabolism ceases following AA administration (Farodoye et al., 2024). In the presence of both morin/ion, the structure of mitochondria and ATP production was significantly improved, indicating a connection to oxidative stress in the damage of mitochondria (Saleh et al., 2024).

**Apoptosis and Autophagy**

Acrylamide increases the level of Bax/Bcl-2, switches on caspase-3 and modulates expression of LC3/Beclin-1 in testicle tissue. That it sets in motion both apoptosis and autophagy — two ways to kill cells. It has been reported that germ cells exhibit increasing caspase-3 activity and signs of apoptosis following exposure to acrylamide for a period of time (Yang et al., 2023; Kucukler et al., 2020). Administering antioxidants simultaneously cured these problems, demonstrating that apoptosis and autophagy are directly associated with oxidative damage to mitochondria (El-Shehawi et al., 2022).

**Table 1:** Oral/dietary *In Vivo* studies on Acrylamide toxicity

| Author (Year) | Model/ Species | AA dose (mg/kg or equivalent) | Duration (days) | Endpoints | Main Findings |
|---|---|---|---|---|---|
| Ajibare et al., 2024 | Rat | 10 | 56 | Sperm quality, Testosterone, FSH, LH, GnRH, MDA, SOD, CAT, Nrf2, HO-1, NF-kB, Bax/Bcl-2 | AA ↓SOD/CAT & hormones; Zinc (1–3) restored redox balance and testicular histology via Nrf2/HO-1 and NF-kB modulation. |
| Gür et al., 2023 | Rat | 38.27 | 21 | MDA, SOD, GSH, Nrf2, Keap-1 (IHC), histology (kidney, testis, brain, heart) | AA ↑MDA and ↓SOD/GSH in multiple organs; Boron (10–20) activated Nrf2/Keap-1 and ameliorated lesions in testis and kidney. |
| Anvari et al., 2020 | Mouse (Mus musculus) | 10 | 35 | Sperm count, motility, viability, chromatin (AO, AB, CMA3), Testosterone | AA ↓sperm parameters & chromatin integrity; Vitamin E (100, IP) restored testosterone and sperm quality. |
| Üremiş et al., 2024a | Rat (prenatal adulthood) | 3 | 84 | Liver/kidney function, OS markers, NF-kB, Akt, Bax/Bcl-xL, Casp-9, histology | Lifelong AA (fetal to adult) caused hepato-renal injury (↑OS, inflammation, apoptosis); Vitamin E ameliorated via Akt/NF-kB and Bax/Bcl-xL modulation. |
| Sedik et al., 2025 | Rat (Sprague–Dawley) | 20 | 27 | cTnI, CK-MB, LDH; cardiac MDA, GSH, SOD; TNF-α/IL-6; Nrf2/HO-1; Bax/Bcl-2; PD-L1; histology/EM | AA caused cardiotoxicity (↑biomarkers, ↑MDA, ↓GSH/SOD, ↓Nrf2/HO-1, ↑TNF-α/IL-6, ↑Bax/↓Bcl-2); cordycepin (10–20) dose-dependently reversed changes. |
| Abd Al Haleem et al., 2022 | Rat (male) | 35 | 56 | Sperm indices, chromatin (AO/AB/TB/CMA3), testosterone, OS markers, NF-kB/p65, occludin | AA induced OS, inflammation and TJ disruption; thymoquinone/capsaicin improved sperm/testosterone, ↓NF-kB, ↑occludin, restored redox. |
| Abdel-Daim et al., 2020 | Rat (Wistar) | 20 | 14 | ALT/AST/ALP; urea/creatinine; 8-oxo-dG, MDA, NO; GSH, GPx, SOD, CAT (liver/kidney/brain) | AA increased hepato-renal-neuro toxicity & OS; thymoquinone (10–20) normalized serum/OS markers and improved tissues dose-dependently. |
| Abd-Elghaffar et al., 2015 | Rat (Sprague–Dawley) | 25 | 28 | Testis OS (MDA/NO, GSH/SOD/CAT), testosterone, testis histology | AA caused testicular OS and lesions; garlic oil (50, twice weekly) partially ameliorated biochemical and histological damage. |
| Abd-Elsalam et al., 2021 | Rat (Wistar, male) | 10 | 60 | Sperm indices, testis OS (LPO, enzymes), testosterone, histology/IHC (PCNA, Casp-3), Bcl-2/Bax expression | AA impaired sperm, ↑LPO, ↓antioxidants, ↓testosterone; Eruca sativa seed extract (100–200) improved semen, restored redox/hormones, modulated Bcl-2/Bax. |
| Abduljalil et al., 2024 | Rat (adult male) | 50 | 21 | Histology & histochemistry of liver/kidney/testis | AA caused multi-organ histological/histochemical damage; Moringa oleifera leaf nanoparticles markedly ameliorated lesions and staining abnormalities. |
| Ahmed et al., 2022 | Rat (male albino) | 5 | 21 | Sperm count/motility/viability; testis GSH/MDA/NO; testosterone/FSH/LH; histology; IHC (p53, Ki-67) | AA reduced sperm quality, ↑MDA/NO, ↓GSH; histo-damage ± Leydig hyperplasia; earthworm extract (300) improved sperm, redox, p53/Ki-67, histology. |
| Li et al., 2022 | Rat (PBPK model) | 0.1–50 | — | Urinary AAMA/GAMA; Hb-AAVal; DNA adducts | PBPK model linked oral intake to biomarkers ($R^2$>0.76); supports internal dosimetry for AA/GA and biomarker interpretation in rats. |
| ALKarim et al., 2015 | Rat (♂/♀ Sprague–Dawley, post-weaning) | ~0.06 | 60; 90 | Reproductive organ weights; fertility; testis/ovary/uterus histology; comet assay | Low-dose AA reduced reproductive organ weights, caused gonadal lesions, sperm abnormalities and ↑DNA damage; impaired fertility outcomes. |
| Alturki et al., 2022 | Rat (male) | 20 | 14 | T, FSH, LH; sperm count/motility/abnormalities; testis GPx/CAT/SOD, MDA; DNA fragmentation; histology | AA impaired hormones, sperm and antioxidants; ↑MDA & DNA damage. Silymarin co-treatment improved redox, endocrine and histological injury. |
| Amirshahrokhi, 2021 | Mouse (male) | 20; 30 | 21 | Colitis indices; colon length; OS (MDA, protein carbonyls, NO), GSH/SOD/CAT; cytokines; NF-kB/iNOS | Chronic AA worsened experimental colitis via ↑OS/inflammation & NF-kB/iNOS; ↑DAI, tissue damage and mortality. |
| Atasever et al., 2025 | Rat (male, lung model) | 50 | 11 | Lung OS (MDA, GSH, SOD, CAT); TNF-α, IL-1β/6, COX-2, iNOS, NF-kB; apoptosis; Nrf2/HO-1; histology | AA induced lung injury (↑OS, inflammation, apoptosis; ↓Nrf2/HO-1). Melatonin (10–20, IP) restored redox, reduced inflammation/apoptosis and improved histology. |
| Banc et al., 2022 | Rat (Wistar) | 0.25 | 28 | AST/ALT; hepatic MDA/TBARS, GSH; SOD/CAT/GPx; histology | AA caused hepatotoxicity & OS; wine polyphenols preserved GSH, restored SOD/CAT, ↓MDA/TBARS and improved liver histology. |
| Baraka et al., 2024 | Rat (male Wistar) | 40 | 28 | GnRH, T, FSH, LH; sperm; 17β-HSD, CYP11A1, CYP17A1; GSH/MDA/NO; NF-kB/TNF-α; histology | AA disrupted HPG axis, steroidogenesis, sperm and redox; apigenin restored hormones, sperm, steroidogenic enzymes and reduced OS/inflammation. |
| Bhuiyan et al., 2023 | Mouse (male) | 45 | 90 | Testosterone; sperm count/motility/morphology; testis/epididymis size; histology | AA reduced testosterone, sperm quality and organ size; Vit C and 5-ASA improved outcomes, with combination most effective. |
| Cakmak et al., 2025 | Rat (Sprague–Dawley) | 38.27 | 10 | Cardiac SOD, CAT, GPx, GSH, MDA; ER-stress (ATF6, PERK, IRE1, GRP78, CHOP); NF-kB, TNF-α, IL-1β, iNOS, COX-2; apoptosis; histology | AA induced cardiac OS, ER-stress, inflammation and apoptosis; morin (50–100) attenuated these and improved histology. |
| Ara et al., 2021 | Mouse (albino) | 20; 40; 80 | 30 | Liver/kidney/testis histology; ALT/ALP/bilirubin; creatinine/urea; testosterone | AA caused multi-organ toxicity; Camellia sinensis extract reduced lesions and improved biochemistry (except at highest AA dose). |
| Daoudi et al., 2025 | Rat (male) | 20 | 21 | $T_3/T_4$; thyroid & testis/epididymis histology; sperm (count/motility/vitality); testosterone; BW gain | AA caused hypothyroidism and thyro-reprotoxicity; Teucrium polium (400) improved hormones, sperm parameters and histology. |
| Dos Santos et al., 2023 | Rat (pre-pubertal → adult) | 2.5; 5 | — | Testis antioxidants (G6PDH, GPx, SOD, CAT, GST), GSH; LPO; protein carbonyls; DNA damage; IBRv2 | Early-life AA impaired adult testicular antioxidant system and ↑carbonyls/LPO; composite toxicity index (IBRv2) increased dose-dependently. |

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|---|---|---|---|---|---|
| Duan et al., 2015 | Mouse (female, ICR) | ~10 | 42 | Oocyte maturation; spindle/chromosomes; ROS/apoptosis; epigenetic marks; litter size | AA reduced oocyte competence and litter size, increased ROS/apoptosis and disrupted spindle organization and epigenetic marks. |
| Edres et al., 2021 | Rat (male) | 50 | 21 | Brain LPO, 8-OHdG, GSH; 5-HT, DA, ACh/AChE; Keap-1/Nrf2/NF-kB; histology | AA induced brain OS, neurotransmitter disruption and NF-kB activation; allicin or melatonin mitigated biochemical and histological damage. |
| El-Beltagi & Ahmed, 2016 | Rat (male) | 20 | 30 | LPO, GSH, SOD/CAT/GPx; serum biochemistry and hormones | AA caused systemic OS and serum changes; quercetin (50–100) restored antioxidant status and serum markers. |
| Elkomy et al., 2018 | Rat (male) | 20 | 21 | ALT/AST; protein/albumin; urea/creatinine; testosterone; MDA/SOD (liver/kidney/testis); histology | AA induced hepato-reno-testicular toxicity; clove oil (100–200) attenuated biochemical alterations and histological injury. |
| Elsayed et al., 2024 | Rat (male) | 20 | 30 | Testosterone/FSH/LH; testis MDA, GSH, SOD, CAT; morphometry; caspase-3 | AA ↓androgens & antioxidants, ↑MDA and apoptosis; CoQ10 and rosuvastatin (each 10) protected, combination most effective. |
| El-Shehawi et al., 2022 | Rat (male) | 20 | 28 | Liver enzymes; MDA/NO; SOD/CAT/GSH; cytokines; Nrf2/HO-1; fibrosis/apoptosis genes; histology | AA induced hepatic OS, inflammation and apoptosis; Taify pomegranate juice restored antioxidants, reduced cytokines/apoptosis and improved histology. |
| Erdemli et al., 2019 | Rat (pregnant → offspring) | 10 | 21 | Offspring testes: hormones, OS, histology (assessed at 8 weeks) | Prenatal AA exposure decreased testosterone and antioxidants, ↑MDA; maternal vitamin E improved SOD/CAT/GSH and preserved testicular structure. |
| Essa et al., 2025 | Rat (male) | 20 | 14 | Liver enzymes; MDA/NO; GSH/SOD/CAT/GPx; IL-6/TNF-α; comet assay; histology | AA caused hepatic OS, inflammation and DNA damage; nano-encapsulated ferulic acid in sesame protein isolate more effective than free form. |
| Famurewa et al., 2024 | Rat (male) | 50 | 14 | Cerebellar AChE; SOD/CAT/GPx; MDA; IL-6/TNF-α/IL-4/IL-10; Nrf2/NF-kB; caspase-3/9; DNA fragmentation; histology | AA caused cerebellar OS, neuroinflammation and apoptosis; thymoquinone (5) improved antioxidants, modulated Nrf2/NF-kB and reduced lesions. |
| Farag et al., 2021 | Rat (male) | 10 | 60 | Sperm indices; testosterone; testis GSH/MDA; Leydig apoptosis; PCNA/caspase-3; CYP11A1, 17β-HSD | AA impaired sperm/testosterone, ↑OS and apoptosis, ↓steroidogenesis; Portulaca oleracea seeds extract (200–400) dose-dependently reversed changes. |
| Farodoye et al., 2024 | Drosophila melanogaster | 25; 50; 100 | 7 | Survival, emergence, locomotion/sleep; mitochondrial OXPHOS; apoptosis genes | AA exposure caused dose-dependent behavioural deficits, mitochondrial dysfunction and apoptosis in flies. |
| Firouzabadi et al., 2022 | Rat (female) | 10 | 56 | Ovarian MDA, GSH, SOD, CAT; caspase-3; histology | AA induced ovarian OS/apoptosis and structural damage; ascorbic acid (200) ameliorated these effects. |
| Gao et al., 2021 | Mouse (female) | 10 | 28 | GV-oocyte morphology; sperm–oocyte binding; IVF/embryo development; ΔΨm; F-actin; autophagy/apoptosis | AA impaired oocyte competence and embryo development via mitochondrial dysfunction, actin disorganization and ↑autophagy/apoptosis. |
| Gao et al., 2023 | Mouse (adolescent male) | 10 | 28 | Testis/epididymis indices; sperm; meiosis markers; DSB markers; histology | AA triggered spermatocyte meiotic arrest via excessive DNA DSBs; resveratrol mitigated DNA damage and restored meiotic proteins. |
| Gupta et al., 2025 | Rat (male) | 40 | 10 | FSH/LH/testosterone; sperm; LPO/GSH/SOD/CAT; lipids; seminal fructose; comet | AA impaired hormones and sperm, ↑LPO and DNA damage; caffeic acid (10–40) dose-dependently restored parameters. |
| Hasanin et al., 2018 | Rat (male) | 10 | 28; 42 | Testis histology/ultrastructure; seminiferous tubule diameter and epithelium | IP AA induced progressive testicular degeneration; oral vitamin E (400/day) preserved testicular structure and reduced damage. |
| Mostafa-Hedeab et al., 2023 | Rat (Wistar male) | 20 | 60 | Sperm count/motility; FSH/LH/testosterone; MDA/CAT/GSH; caspase-3; histology; StAR/CYP11A1/3β-HSD/17β-HSD mRNA | AA caused testicular OS and apoptosis; green-synthesized ZnO NPs from Moringa oleifera restored antioxidants, hormones and steroidogenic gene expression. |
| Yang et al., 2021 (in vivo part) | Rat brain | 20 | 30 | Mitochondrial ROS; PGC-1α, TFAM, Mfn2, Opa1; mtDNA expression | AA induced mitochondrial ROS, disturbed biogenesis/dynamics and mtDNA expression; mitoquinone mitigated these alterations. |
| Zhang et al., 2022a | Rat (SD male) | 20 | 28 | DNA adducts in liver/kidney/urine; MDA; GSH/SOD; repair genes; histology | AA increased DNA adducts and OS; catechins reduced adducts (~60%), restored GSH/SOD and DNA-repair genes. |
| Khyoon & Abdulwahid, 2024 | Khyoon & Abdulwahid, 2024 | Khyoon & Abdulwahid, 2024 | Khyoon & Abdulwahid, 2024 | Khyoon & Abdulwahid, 2024 | Khyoon & Abdulwahid, 2024 |

**Table 2:** Intraperitoneal *In Vivo* experimental studies on Acrylamide toxicity

| Author (Year) | Model/Species | AA dose (mg/kg) | Duration (days) | Endpoints | Main Findings |
|---|---|---|---|---|---|
| Trigg et al., 2021 | Mouse | 100 (single IP dose) | 3–24 (sampling) | Epididymal proteome, sperm sncRNA (miRNA, piRNA), embryo transcriptome | Single IP AA dose altered epididymal proteome and sperm sncRNA cargo; embryos showed transcriptome dysregulation and developmental defects. |
| Hasanin et al., 2018 | Rat (male) | 10 | 28; 42 | Testis histology & ultrastructure; seminiferous tubule morphometry | IP AA caused progressive testicular degeneration; high-dose oral vitamin E protected testicular architecture. |
| Ligina et al., 2022 | Fish (*Anabas testudineus*) | $LC_{50}$ = 132; sub-lethal 13.2 | 24–96 | Hematology, SOD, CAT, GR, $H_2O_2$, MDA, behaviour | Sub-lethal AA decreased RBC, Hb, PCV and antioxidant enzymes; increased WBC, $H_2O_2$, MDA and behavioural stress responses. |

**Table 3:** *In Vitro* experimental studies on Acrylamide toxicity

| Author (Year) | Cell / In vitro model | AA dose (μM)* | Duration (h) | Endpoints | Main Findings |
|---|---|---|---|---|---|
| El-Sheikh et al., 2025 | Bovine oocytes (IVM) | 250–1000 | ≤24 | Cleavage/blastocyst rates; F-actin; DNA damage; LC3A/B, ATG7; DPPA3, EZH1/2 | ACR/GLY reduced embryo development and increased DNA damage, apoptosis, autophagy and dysregulated epigenetic genes. |
| Aydin, 2018 | Mouse Leydig (TM3), Sertoli (TM4) | 10–1000 | 24 | Viability/LDH; ROS/LPO; SOD/CAT/GSH; steroidogenic & apoptotic genes | AA (and GA) increased ROS/LPO, reduced antioxidants/viability, dysregulated steroidogenesis and ↑pro-apoptotic gene expression. |
| Calmaz et al., 2025 | Leydig (TM3) | 1000 | 24 | Viability/LDH; ROS/LPO; GSH, SOD/CAT/GPx; apoptosis; Trp53, Casp3, Bax, Bcl2 | Luteolin antagonized AA toxicity: ↑viability, ↓ROS/LPO, preserved antioxidants and shifted apoptotic gene balance toward survival. |
| Deng et al., 2022 | U87-MG human astrocytoma | ~1000 | 24 | ROS, MDA, $Ca^{2+}$; ΔΨm; LC3II/I, p62; caspase-9/3; Bax/Bcl-2; NF-kB | AA caused ROS-mediated mitochondrial dysfunction, blocked autophagic flux and induced intrinsic apoptosis with NF-kB activation. |
| Hong et al., 2021 | BRL-3A rat hepatocytes | 5000 | 24 | ROS/MDA/GSH/SOD; ER-stress markers; MAPKs; apoptosis | AA induced OS, ER-stress and apoptosis; rosmarinic acid pretreatment suppressed OS/ER-stress/MAPK activation and reduced apoptosis. |
| Pan et al., 2018 | PC12 neuronal cells | ≤1000 | 24 | ROS, MDA, GSH; TNF-α, IL-6; Nrf2, NF-kB; MAPKs | AA ↑ROS/MDA and activated MAPKs, driving Nrf2/NF-kB crosstalk; NAC reduced ROS/TNF-α and restored Nrf2. |
| Pan et al., 2017 | PC12 neuronal cells | ≤1000 | 24 | ROS, MDA, ΔΨm, Bax/Bcl-2, cytochrome c, caspase-3/9; MAPKs; Nrf2 | AA increased ROS and mitochondrial dysfunction, triggering intrinsic apoptosis; balance between MAPKs and Nrf2 determined survival. |
| Yuan et al., 2024 | PC12 cells | 250–1000 | 24 | ROS, $Ca^{2+}$, GRP78/CHOP/p-eIF2α; ΔΨm; caspase-3; apoptosis | Combined AA + elaidic acid induced synergistic ER-stress and mitochondrial apoptosis stronger than either alone. |
| Mori et al., 2022 | In vitro DNA system | μM range | — | DNA cleavage, 8-oxo-dG, Cu(II)/Cu(I) | Acrylohydroxamic acid (putative AA metabolite) with Cu(II) generated ROS ($H_2O_2$) and induced oxidative DNA damage. |

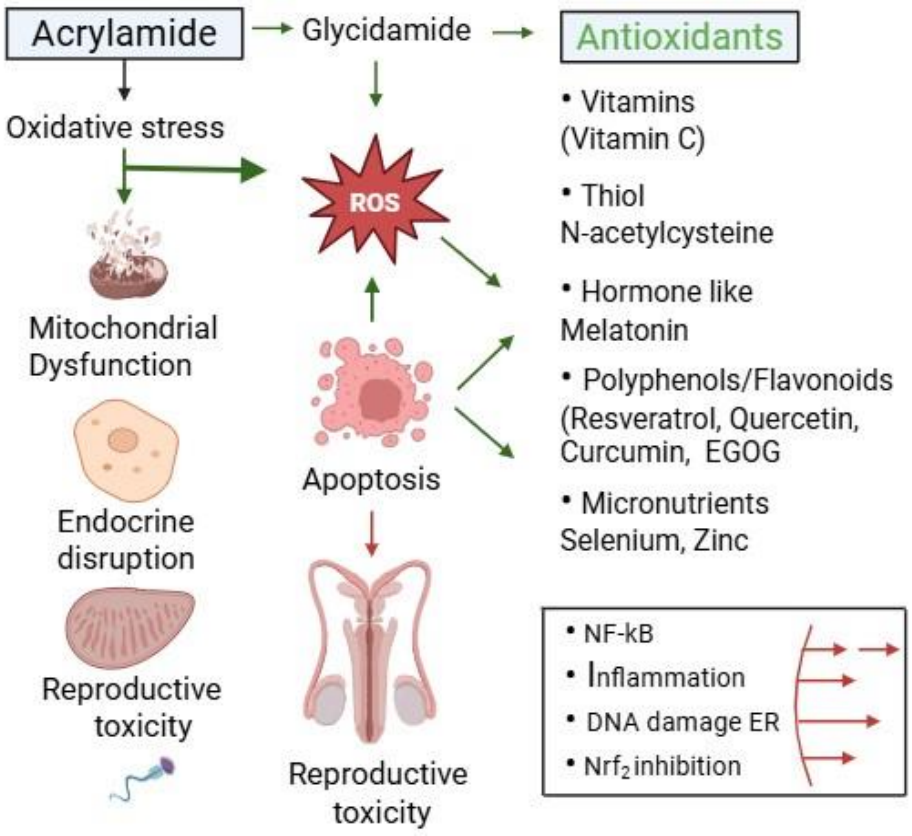


**Fig. 1:** Correlation network depicting relationships between oxidative stress and molecular defense markers. Green edges show positive correlations ($r > 0.7$), red edges negative ($r < -0.7$). Node size is proportional to correlation magnitude.

**Endocrine Disruption**

AA modifies the level of hormones by decreasing the blood testosterone and in highly paired gonadotrophin release (LH and FSH). This occurs because it affects the functioning of Leydig and Sertoli cells (Sciorio et al., 2024; Ali et al., 2025). In the testes, AA has been observed to decrease expression of steroidogenic enzymes such as 3β-HSD, and 17β-HSD, suggesting it interferes with steroids biosynthesis in general (Farag et al., 2021). Treatment with selenium or zinc, however, restored testosterone levels to a control condition and prevented AA-induced alterations in Leydig cell appearance suggesting altered hormone homeostasis is a prominent sign of AA damaging effects (Yildirim et al., 2022).

**DNA and Chromatin Damage**

Glycidamide can react with DNA to produce cytotoxic and genotoxic effects (such as oxidative damage) to proteins, lipids, and DNA including gene mutation (Deng et al., 2022; Singh et al., 2024). Mouse and human sperm studies with acrylamide (AA) also demonstrated increased comet tail lengths and more chromosomal defects, suggesting that it can directly affect DNA (Nixon et al., 2012; Katen et al., 2016). The degree of DNA fragmentation

was found to be correlated with elevation in MDA content and reduction in GSH level, which indicates that oxidative stress induced genotoxicity (Dhillon et al., 2024).

**Inflammation and Endoplasmic-Reticulum Stress**

The AA activates the NF-kB [a protein that regulates inflammation] and elevates the level of TNF-α, IL-6 (inflammatory factors) in testes (Soliman et al., 2023). These signals enhance oxidative injury and initiate cell death. Concomitantly, markers of ER stress such as GRP78/BiP and CHOP are induced indicating that AA deranges the regular pathway of unfolding. This leads to cell death through the PERK–CHOP pathway (Yuan et al., 2024). Furthermore, they have become at least partially associated with AA's toxic effects and the elevation of these inflammation-/ER stress-associated proteins were already diminishing upon anti-oxidative treatment (Hong et al., 2021).

**Nrf2/Keap1-Signaling Impairment**

Acrylamide exposure might suppress Nrf2/Keap1, leading to a lower level of Nrf2 in the nucleus. As a consequence, the expression of protective enzymes including HO-1 and NQO1 is reduced (Cengiz et al. 2024). Cells have less capacity to defend themselves from oxidative harm when Nrf2 signaling is reduced, which can increase the facilitated presence of damage in cells. Nrf2 reactivation may also be achieved using antioxidants as zinc (Mao et al., 2025) and polyphenols, which HO-1 releases could inhibit AC/ROS-induced oxidative stress and cell death induced by acrylamide (Ajibare et al., 2024).

**Integrated Mechanistic Pathway**

Based on the current evidence, when CYP2E1 metabolizes acrylamide to glycidamide, reactive oxygen species (ROS) are produced and these subsequently lead to induction of oxidative stress and mitochondrial injury. These early problems subsequently lead to apoptosis, inhibit steroid synthesis, DNA damage and produce inflammation and endoplasmic reticulum (ER) stress (Katen et al., 2017; Zhang et al., 2025b). Simultaneous inhibition of Nrf2/Keap1 signaling further increases cellular susceptibility while antioxidant co-treatments restore redox homeostasis and impend testicular function (Abd Al Haleem et al., 2022).

**Evidence in Animals and Cells**

**Rodents in vivo (acute vs sub-chronic)**

Across mouse and rat models, acrylamide (AA) or glycidamide (GA) exposures produced a consistent pattern of male-reproductive injury encompassing depressed sperm indices, reduced relative testis/epididymis weights and characteristic histopathology of the seminiferous epithelium (Gür et al., 2023; Seify et al., 2024). In rats treated with acrylamide (10mg/kg for 60 days), epididymal sperm motility, viability, normal sperm percentage and sperm count was significantly reduced, while the percentage of abnormal sperm increased, concurrent with degeneration of the seminiferous epithelium and germ cell loss (Farag et al., 2021). Saleh et al. (2024) demonstrated that rats orally exposed to acrylamide at 20mg/kg/day for 28 days exhibited significant oxidative stress (↑MDA, ↓GSH, ↓GPx) alongside Leydig cell degeneration, seminiferous epithelial disorganization, and tubular atrophy. Trigg et al. (2021) demonstrated that glycidamide (GA), the CYP2E1-mediated oxidative metabolite of acrylamide (AA), exhibits greater reproductive and genotoxic potency than AA; in mice, GA exposure produced stronger DNA adduct formation, oxidative imbalance, and spermatogenic disruption at equal or lower nominal doses, confirming bioactivation-dependent toxicity. Antioxidant co-treatments (vitamin E, zinc and polyphenols) partially restored sperm quality, normalized redox enzymes and mitigated tubular pathology functionally linking oxidative stress with structural and endocrine injury in vivo (Anvari et al., 2020; Ajibare et al., 2024).

**In vitro Sperm and Testicular Cells (Concentration–Effect Features)**

Rodents or human sperm incubated with AA exhibited concentration-dependent increases in ROS, loss of plasma-membrane integrity, mitochondrial depolarization, and declines in progressive motility and viability; effects typically became evident from ≥0.1–1mM AA with steeper deterioration at higher millimolar levels and longer incubations (Gao et al., 2023; Üremiş et al., 2024). GA produced comparable or stronger effects at lower concentrations than AA, consistent with its higher genotoxic reactivity (Eisenbrand, 2020). Testis-derived somatic cell lines (Leydig/Sertoli) and primary germ-cell cultures exposed to AA/GA showed ROS surges, Δψm collapse, cytochrome-c release, Bax/Bcl-2 shift, and caspase-3 activation; autophagy markers (LC3, Beclin-1) were also perturbed, indicating stress-response remodeling (Wang et al., 2023; Ma et al., 2025).

**Dose-response and NOAEL Hints (Oxidative-stress and Function-linked Thresholds)**

Across animal studies, dose-response patterns are evident for both oxidative biomarkers and functional sperm outcomes. Many sub-chronic rat/mouse experiments report the onset of measurable oxidative stress and early sperm impairment beginning around ≥2–5mg/kg/day oral AA, with progressively larger effects through 10–30mg/kg/day (Ivanski et al., 2020). Moradi et al. (2025) reported that acute administration of acrylamide at 50mg/kg resulted in a marked decline in sperm motility, viability, and count. GA, when administered directly, often produced comparable injury at lower nominal doses than AA, supporting the metabolic-activation model (Gupta et al., 2023; Singh et al., 2024). While formal no observed adverse effect level vary by species/strain, exposure duration, and endpoint sensitivity, several sub-chronic investigations suggest little or no effect on sperm quality and testicular histology below ~1–2mg/kg/day, with oxidative and reproductive endpoints becoming consistently altered above this band; however, study heterogeneity (diet, age, assay methods) warrants cautious interpretation (Timmermann et al., 2021; Yu et al., 2022; Estevan et al., 2025). In vitro, benchmark-like concentrations for detectable ROS elevation and motility decline frequently fall in the 0.1-1mM AA range over a few hours, with GA active at lower micromolar–submillimolar

levels, again reflecting potency differences (Gao et al., 2023; El-Sheikh et al., 2025). Antioxidant rescue experiments demonstrate graded protection (e.g., partial normalization of MDA and SOD/CAT/GPx and recovery of motility) that tracks with dose, timing, and redox capacity of the agent, lending mechanistic weight to oxidative stress as the proximate driver of functional decline (Calmaz et al., 2025; Kumar et al., 2025).

Rodent in vivo and cell-based studies converge on a reproducible injury signature oxidative stress, mitochondrial dysfunction, apoptosis, and chromatin/DNA damage that scales with exposure intensity and duration, is more severe with GA than AA, and is partially reversible with antioxidants that restore Nrf2-linked defenses (Zhang et al., 2023; Rajeh, 2024; Atasever et al., 2025; Cakmak et al., 2025).

**Human Evidence**

Epidemiology/Biomonitoring (Hb Adducts, Semen Quality, Hormones). In humans, acrylamide exposure is routinely quantified via hemoglobin adduct biomarkers specifically the N-terminal valine adducts of acrylamide (HbAA) and its epoxide metabolite glycidamide (HbGA) (Table 4). Across approximately 65 biomonitoring studies encompassing over 25,000 individuals worldwide, HbAA and HbGA concentrations vary widely according to smoking status, diet, and occupational exposure, with smokers and industrially exposed workers displaying the highest adduct burdens. Background levels in general populations typically range from the tens to low hundreds of pmol/g Hb, reflecting dietary and environmental sources, whereas occupationally exposed individuals and smokers often exceed several hundred pmol/g Hb. These adducts thus serve as robust, time-integrated biomarkers of long-term internal acrylamide exposure (Hung et al., 2021; Narii et al., 2023; Li et al., 2025; Zhang et al., 2025b).

One occupational biomonitoring study measured HbAA at ~54pmol/g in workers handling polyacrylamide gels, compared to ~31pmol/g in non-smoking controls, and markedly higher levels (~116pmol/g) in smokers, correlating with number of cigarettes smoked. These findings confirm that both occupational and lifestyle exposures contribute significantly to internal dose.

Despite robust exposure biomarker data, few human studies have directly correlated Hb adduct levels with male reproductive endpoints. One in vitro human sperm exposure experiment exposed semen samples ex vivo to 0.5, 1.0, and 2.0mM AA for 2 hours and observed significant decreases in sperm motility, viability, and total antioxidant capacity (TAC), along with increased ROS and MDA levels While this is not an epidemiologic correlation between Hb adduct status and fertility in living men, it offers proof-of-concept that human sperm respond to AA in a dose-dependent manner (Kashani et al., 2021; Hung et al., 2021; Wang et al., 2023).

In the Swedish Riksmaten Adolescents cohort, HbAA and HbGA hemoglobin adducts were quantified in approximately 600 samples using LC–MS/MS, while dietary acrylamide exposure was estimated from recall data (fried potatoes, crisps, bread). Individuals reporting higher intake frequencies exhibited higher adduct levels, particularly for fried foods, though associations were weak and often lost statistical significance after Bonferroni correction (Vryonidis et al., 2024). Similar findings were reported in NHANES biomonitoring and European meta-analyses, where HbAA/HbGA adducts showed modest diet-related variation but strong influences of smoking and occupational exposure (Buyukdere and Akyol, 2024; Gan et al., 2025).

So far, no large-scale prospective cohort or case-control human study has robustly linked Hb adduct levels of AA/GA to semen quality (sperm count, motility, morphology) or hormonal profiles (testosterone, LH, FSH). Some smaller cross-sectional fertility clinic studies have attempted correlations between exposure markers (smoking, dietary proxies) and semen parameters, but these are subject to confounding and limited design strength (Rajczewski et al., 2023; Baraka et al., 2024).

**Limitations and Gaps in Human Evidence**

One major limitation is confounding: dietary sources of acrylamide overlap with multiple factors relevant to fertility (e.g. high-fat foods, processed foods, glycemic load), and co-exposures to other heat-generated toxicants (e.g., heterocyclic amines, polycyclic aromatic hydrocarbons) complicate attribution. Smoking is itself a strong reproductive toxicant and a major source of acrylamide, making disentangling the specific effect of AA challenging.

Another limitation is sample size and statistical power: many biomonitoring studies focus on hundreds rather than thousands of subjects, limiting sensitivity to detect small-to-moderate associations with reproductive endpoints. Also, cross-sectional designs predominate, which cannot establish temporality or causality. Without longitudinal data or pre-to-post intervention designs, it is hard to establish that higher Hb adduct levels precede declines in sperm quality.

It is also difficult to accurately measure things. Variability among people, laboratory errors and the length of time chemicals remain in the body (for example, Hb adducts reveal exposure over approximately 120 days the red blood cell life span) can make it difficult to see links, particularly if exposures fluctuate widely. And many studies do not measure both reproductive health and hormone levels at the same time, so it is difficult to link exposure to a particular substance with fertility outcomes. The problems associated with this pattern have left us with mostly indirect evidence from people, which provides some clues but is not direct proof. We very much require big, long studies or trials that are actually looking to test ways of making things better and also link HbAA/HbGA levels with semen/hormone changes. To obtain stronger evidence, future studies would need to track groups of men of reproductive age over time, or compare fertile men with those who have fertility issues. These studies should periodically measure Hb adducts, semen, and hormone levels and also consider the effects of diet, smoking habits, age, weight, and other exposures.

**Table 4:** Summary of Human Studies Assessing Acrylamide Exposure, Biomarkers, and Associated Outcomes

| Author (Year) | Model / Population | AA Dose / Exposure | Duration | Route / Assessment | Endpoints | Main Findings |
|---|---|---|---|---|---|---|
| Kenwood et al., 2022 | Humans (U.S. NHANES adults ≥ 18 y) | Internal biomarker 2-CaEMA(ng/mL urine) | Cross-sectional (2011–2016) | Biomonitoring (urine) | Urinary 2-CaEMA by smoking status and CPD dose | Smokers had markedly ↑ 2-CaEMA vs non-smokers (positive dose-response with CPD); smoking major AA source in population. |
| Wan et al., 2022 | Humans (Chinese middle-aged and elderly, n = 1,272) | Estimated dietary intake ≈ 8.9μg/day; urinary biomarkers (AAMA, GAMA, iso-GAMA) | Cross-sectional (one time point) | Dietary + biomarker analysis | Dietary AA intake, urinary metabolites, ML model prediction | Machine-learning model (SVR) linked urinary metabolites to dietary AA intake (R ≈ 0.4); demonstrates exposure assessment feasibility. |
| Pedersen et al., 2022 | Humans (biomonitoring review; ~27,966 across 19 countries) | Hb-AA 3–210pmol/g Hb in non-smokers; higher in smokers/occupational cohorts | Biomarker window ≈120 days | Blood Hb adducts (AA/GA) | Hb-AA/Hb-GA levels; exposure variation; methods/gaps | 86-study synthesis; wide inter-individual range; smokers/occupational highest; calls for standardized, longitudinal designs. |
| Vryonidis et al., 2024 | Adolescents in Sweden (~n=600) | Median Hb-AA ~34 pmol/g Hb (range 14–225); intake slightly < EFSA adolescent estimate | Cross-sectional; biomarker window ≈120 days | Dietary intake + Hb adducts | Hb-AA/Hb-GA; GA/AA ratio (~1.4 median); demographics | Widespread exposure; no strong differences by age/sex/residence; adduct-derived intake broadly aligned with dietary recall. |
| Li et al., 2022 | Humans (PBPK model) | 0.0005–0.020mg/kg (modelled human intake range) | Various (datasets) | Oral (dietary); biomonitoring | AAMA/GAMA, AAVal, DNA adducts | PBPK connected intake to biomarkers across ranges; improved parameterization for translating intake ↔ internal dose. |
| Zhang et al., 2025a | Humans (multi-omics + MR) | Dietary AA exposure (estimated; not a fixed mg/kg/day) | Cross-sectional + MR | Dietary exposure proxies; omics biomarkers | Depression risk; SIRT3/mitochondrial oxidative injury | Higher AA linked to depression; evidence for SIRT3-mediated mitochondrial oxidative injury pathway (MR supports causality). |
| Chu et al., 2020 | Human males (NHANES 2003–04, n=468) | HbAA/HbGA adducts (biomonitoring) | Cross-sectional | Blood adducts; serum hormones | Inhibin B, SHBG, AMH; T, E2, ADTG | n(HbAA) ↑Inhibin B & SHBG; ln(HbGA) ↑AMH; no sig. link to T/E2/ADTG. |
| Duke et al., 2018 | U.S. NHANES 2003–04 | HbAA/HbGA (pmol/g Hb) | Cross-sectional | Biomonitoring + diet/lifestyle | Adducts vs diet (fried potatoes, chips, coffee), smoking, BMI, demographics | Dietary AA and behaviors significantly associated with HbAA/HbGA; smoking/demographics influence levels. |
| Esposito et al., 2022 | Habitual smokers (CC vs HTP) | Smoke AA per stick: CC ~235–897ng; HTP ~99–187 ng | Cross-sectional | Inhalation; emission quant + intake/MOE/ILCR | Emissions; exposure; risk metrics | CC = major AA source; HTP lower but non-zero; carcinogenic risk elevated with CC at typical CPD; neurotoxic MOE low concern. |
| Filippini et al., 2022 (meta-analysis) | General population | Dietary intake (~μg/day) | Median FU ~14.9 yrs | Dietary assessment | Site-specific cancer incidence | Overall null association diet-AA vs cancers; ↑lung cancer risk in smokers. |
| Gan et al., 2025 | U.S. adults (NHANES 2013–16) | Biomarkers: HbAA/HbGA, urinary AAMA | Cross-sectional | Biomonitoring | Sleep duration/trouble sleeping; inflammation index | Higher AA biomarkers associated with greater odds of short sleep; stronger in smokers; linked with higher systemic inflammation. |
| Poteser et al., 2022 | Children & Adults (10 EU countries, n≈5500) | Biomarkers (HbAA/HbGA) | 2001–2021 | Biomonitoring (LC–MS/MS) | Temporal trends & regulatory impact | Exposure ↑ 2001–2017 then ↓ post-2018 (EU food-regulation effect). |
| Vryonidis et al., 2024 | Swedish adolescents (n = 600) | Dietary AA ≈ 0.3μg/kg bw/day | Cross-sectional (2016–17) | HbAA/HbGA LC–MS/MS + diet recall | Correlation diet–biomarker; smoking effect | Median HbAA 34 pmol/g Hb; smokers +76 pmol/g; diet (main driver). |
| Zhang et al., 2025b | Human multi-omics & MR datasets | Dietary AA exposure | Cross-sectional (omics integration) | Computational (MR, transcriptomics) | SIRT3/SOD2 axis, JUN/PTK2 genes in depression | AA → SIRT3 ↓ → ↑ROS, mitochondrial injury → depression pathways. |
| Wan et al., 2022 | Adults (n≈1200, China) | Dietary AA intake & HbAA/HbGA biomarkers | Cross-sectional | Diet survey + LC–MS/MS | ML prediction of exposure | Random Forest model accurately predicted internal dose ($R^2$ ≈ 0.8). |
| Chu et al., 2020 | NHANES 2003–2004 (468 males) | HbAA, HbGA | Cross-sectional | Biomonitoring (LC-MS/MS) | Inhibin B, AMH, SHBG, T, E2, FSH, LH | HbAA ↑ Inhibin B & SHBG; HbGA ↑ AMH; no T/E2 change → subtle endocrine perturbation. |
| Poteser et al., 2022 | EU population (~5500) | HbAA/HbGA | 2001–2021 | Biomonitoring | Temporal trends | Post-2018 ↓ exposure with EU regulation. |
| Vryonidis et al., 2024 | Swedish adolescents (n = 600) | Dietary intake | Cross-sectional | HbAA/HbGA LC-MS/MS | Diet vs biomarker correlation | Median HbAA 34 pmol/g Hb; smokers +76 pmol/g. |
| Zhang et al., 2025a | Multi-omics datasets | Dietary AA exposure | Cross-sectional | Computational MR & omics | Depression markers (SIRT3/SOD2) | AA ↓ SIRT3 → mitochondrial ROS ↑ → depression risk. |

## Antioxidant Interventions: What Works, Where and How

### Vitamins (C and E): Lipid Peroxidation ↓, GSH Recycling ↑

Vitamins E and C are classical antioxidants that suppress lipid peroxidation and regenerate cellular glutathione. Vitamin E, when co-treated with AA in rats has significantly decreased the level of MDA and TBARS, as well as reversed the normal activity of SOD, CAT, GPx and GSH (Erdemli et al., 2019). Moreover, when vitamin E was applied in combination with 5-aminosalicylic acid, the direct protection for rats was improved with redox indices that became comparable to those of control rats, and there were no more structural disorders observed in their testis than those treated with any compound alone (Rajeh and Khayyat, 2017). The addition of vitamin C to the mix also enhanced recovery of antioxidant enzymes and

stabilized cell membranes, suggesting that these vitamins help regulate lipid peroxidation and protect the health of sperm cells (Davoudimoghadam et al., 2022).

**Thiols (N-acetylcysteine, NAC): GSH Replenishment and Detoxification**

NAC acts as a glutathione precursor and conjugating thiol that detoxifies AA and glycidamide intermediates. Lab tests showed that NAC boosted levels of GSH in the liver and testes, lowered ROS, and lessened cell damage caused by AA exposure (Martin et al., 2024). NAC helped restore Δψm and lessened DNA breakdown in germ cells by directly binding to harmful substances and indirectly improving detoxification that relies on glutathione, which proves how it helps balance oxidation and reduction in the body (Saeed and Ibrahim, 2025).

**Hormone-like Molecules (Melatonin): Mitochondrial Targeting and Nrf2 Activation**

Melatonin seems to work in two ways; it acts as an antioxidant and also helps regulate hormones. Studies in rats given AA showed that melatonin made the membranes of mitochondria more stable, stopped cytochrome c from leaking out, and increased the production of Nrf2 and HO-1, while decreasing Bax and caspase-3 (Karna et al., 2020; Ajayi et al., 2025). Similar studies found that melatonin brought testosterone levels back to normal and made sperm better by protecting the function of Leydig cells and lowering oxidative stress in mitochondria (Moradi et al., 2021).

**Polyphenols and Flavonoids (Resveratrol, Quercetin, Curcumin, EGCG): ROS Scavenging and Anti-inflammatory Modulation**

Polyphenols are strong antioxidants that influence Nrf2 and NF-kB pathways. Quercetin lessened MDA and ROS production in testes treated with AA, and it also boosted sperm count and movement (Uthra et al., 2017). Green tea catechins, specifically EGCG, brought back normal testosterone levels and testicular structure after 90 days of AA exposure, showing they help lessen oxidative stress (Yassa et al., 2014). Curcumin and resveratrol had similar antioxidant and cell-protecting effects in AA models and related situations by helping Nrf2 move into the nucleus and blocking NF-kB from activating (Khyoon and Abdulwahid, 2024; Zhang et al., 2024).

**Micronutrients (Selenium, Zinc): GPx Activation and Steroidogenic Support**

Selenium is a crucial component for glutathione peroxidase to work properly, and zinc helps keep cell membranes strong and aids in the function of enzymes that produce steroid hormones. Giving selenium as a supplement stopped oxidative damage caused by AA, brought back GPx activity, and lowered signs of cell death in rat testicles (Sengul et al., 2021; Yildirim et al., 2024). Adding zinc at the same time made sperm move better, brought testosterone levels back to normal, and greatly increased the levels of Nrf2 and HO-1, showing that it can change both antioxidant and hormone pathways (Ajibare et al., 2024).

**Bioenergetic Compounds (Coenzyme Q10, L-Carnitine): Mitochondrial Restoration and Sperm Motility**

In rats with induced AA, giving them Coenzyme Q10 helped their mitochondria work better, lowered the buildup of harmful ROS, and kept their testosterone levels up, which protected the structure of their testicles (Saleh et al., 2017; Elsayed et al., 2024). L-carnitine also made sperm swim better, reduced MDA levels, and boosted overall antioxidant power in models where reproductive systems were harmed, suggesting it helps move fats in mitochondria and supports energy use (Zamani et al., 2018).

**Plant Extracts: Broad Spectrum Phytotherapy**

Some plant-based substances have shown powerful antioxidant and anti-inflammatory effects against toxicity caused by AA. For example, green tea extract (from Camellia sinensis) helped reverse tissue damage and restore normal testosterone levels in rats that had been exposed to AA for a long time (Elkomy et al., 2018; Ara et al., 2021). Silymarin helped protect testicular tissue by boosting antioxidant enzymes and reducing cell death (Alturki et al., 2022). Other extracts, such as Nigella sativa, propolis, and grape-seed polyphenols, have shown similar effectiveness, but their use is limited because the number of active compounds they contain isn't consistent (Kunnel et al., 2019; Abd Al Haleem et al., 2022).

**Nano-delivery Systems: Enhanced Bioavailability and Testicular Targeting**

Antioxidants in nano form are becoming popular because they can get into tissues more easily and last longer. Zinc-oxide nanoparticles made from Moringa oleifera extract were shown to fix oxidative and hormonal problems caused by AA, and they also reduced testicular cell death in rats (Mostafa-Hedeab et al., 2023). Polyphenols and vitamins in liposome and nanoemulsion forms have also been shown to be absorbed better and work as antioxidants more effectively, but it's still important to study how safe they are over time and where they go in the body (Essa et al., 2025; Manzoor et al., 2025).

**Timing: Prophylactic vs. Therapeutic and Reversibility**

The most successful treatments were preventative, meaning they were given at the same time as, or even before, exposure to AA and they stopped the initial rush of oxidative stress (Abdel-Daim et al., 2020). Treatments given after exposure, like melatonin or eugenol, also helped to undo some of the damage to tissues and hormones (Ozturk et al., 2023). Studies on recovery show that the balance of oxidation and reduction, as well as sperm health, can slowly improve with ongoing antioxidant treatment (Table 5), suggesting that some of the damage can be reversed after exposure stops (Ahmed et al., 2025).

**Table 5:** Antioxidant Interventions and Mechanistic Targets

| Author (Year) | Compound / Class | Dose & Route | Model | Main Outcomes | Mechanistic Target |
|---|---|---|---|---|---|
| Ajibare et al., 2024 | Zinc (trace element) | 1 & 3mg/kg PO | Rat | ↑Nrf2/HO-1, ↓NF-kB/Bax, ↑Bcl-2, ↑SOD/CAT, ↓MDA | Nrf2/HO-1 activation, NF-kB suppression, anti-apoptotic |
| Gür et al., 2023 | Boron (Boric acid) | 10 & 20mg/kg PO | Rat | ↓MDA, ↑SOD/GSH, improved renal and testicular histology | Nrf2/Keap-1 pathway activation |
| Anvari et al., 2020 | Vitamin E | 100mg/kg IP | Mouse | ↑Sperm count & motility, ↑Testosterone, ↓Chromatin damage | Lipid peroxidation inhibition, antioxidant regeneration |
| El-Sheikh et al., 2025 | ACR + Glycidamide (with vitamin E co-exposure) | 250–1000μM in IVM medium | Bovine oocytes | ↑Blastocyst formation, ↓Apoptosis and Autophagy genes after antioxidant addition | Autophagy and epigenetic pathway normalization |
| Ligina et al., 2022 | N/A (no antioxidant) | 13.2μg/L AA sub-lethal (waterborne) | Fish (Anabas testudineus) | ↓SOD/CAT, ↑MDA, hematologic disruption | Oxidative stress induction via ROS overload |
| Mori et al., 2022 | Acrylohydroxamic acid (AA metabolite) | μM range (in vitro DNA system) | DNA model | ↑8-oxo-dG formation, DNA strand breaks | Cu (II) → Cu(I) redox cycle and ROS generation |
| Üremiş et al., 2024b | Vitamin E | 100mg/kg bw/day PO | Rat (prenatal→adult) | Improved liver/kidney function; ↓OS/Inflammation/Apoptosis | Akt/NF-kB modulation; Bax/Bcl-xL balance |
| Sedik et al., 2025 | Cordycepin | 10 & 20mg/kg PO | Rat (heart) | ↓cTnI/CK-MB/LDH; ↓MDA; ↑GSH/SOD; ↑Nrf2/HO-1; ↓TNF-α/IL-6; ↓Bax/↑Bcl-2; histology rescued | Nrf2/HO-1 activation; anti-inflammatory; anti-apoptotic |
| Abd Al Haleem et al., 2022 | Thymoquinone; Capsaicin | 15mg/kg; 10 mg/kg | Rat (testis) | ↑Sperm indices/testosterone; ↓NF-kB; ↑occludin; restored redox | Antioxidant; anti-inflammatory; tight-junction integrity |
| Abdel-Daim et al., 2020 | Thymoquinone | 10 & 20mg/kg PO | Rat (liver/kidney/brain) | Normalized ALT/AST/ALP, urea/creatinine; ↓8-oxo-dG/MDA/NO; ↑GSH/GPx/SOD/CAT | Antioxidant/anti-oxidative damage |
| Abd-Elghaffar et al., 2015 | Garlic oil | 50mg/kg twice weekly, PO | Rat (testis) | ↓MDA/NO; ↑GSH/SOD/CAT; improved histology | Antioxidant/anti-inflammatory |
| Abd-Elsalam et al., 2021 | Eruca sativa seed extract | 100 & 200mg/kg PO | Rat (testis) | Improved semen; ↓LPO; ↑antioxidants/testosterone; modulated PCNA/Casp-3; ↑Bcl-2/↓Bax | Anti-oxidative; anti-apoptotic (Bcl-2/Bax) |
| Abduljalil et al., 2024 | Moringa oleifera leaf nanoparticles | 50mg/kg | Rat (liver/kidney/testis) | Ameliorated histological & histochemical lesions | Nano-antioxidant; tissue protection |
| Ahmed et al., 2022 | Earthworm methanolic extract | 300mg/kg PO | Rat (testis) | ↑Sperm count/motility/viability; ↓MDA/NO; ↑GSH; improved p53/Ki-67 & histology | Antioxidant; anti-apoptotic |
| Alturki et al., 2022 | Silymarin | 100mg/kg | Rat (testis) | Restored T/FSH/LH; ↑sperm metrics; ↑GPx/CAT/SOD; ↓MDA; ↓DNA damage; histology better | Antioxidant; anti-apoptotic |
| Atasever et al., 2025 | Melatonin | 10 & 20mg/kg IP | Rat (lung) | ↓MDA/cytokines/apoptosis; ↑GSH/SOD/CAT; ↑Nrf2/HO-1; histology improved | Nrf2/HO-1 activation; NF-kB suppression |
| Baraka et al., 2024 | Apigenin | 10 & 20mg/kg PO | Rat (HPG/testis/brain) | Restored HPG hormones & sperm; ↑17β-HSD/CYP11A1/CYP17A1; ↓MDA/NO; ↓NF-kB/TNF-α | Steroidogenesis up-regulation; anti-inflam/oxidant |
| Bhuiyan et al., 2023 | Vitamin C + 5-ASA | 100mg $kg^{-1}$; 0–100 mg $kg^{-1}$ PO | Mouse (repro) | Improved T, sperm, organ metrics & histology vs AA | Antioxidant + anti-inflam synergy |
| Cakmak et al., 2025 | Morin | 50 & 100mg/kg PO | Rat (heart) | ↓OS/ER-stress/inflammation/apoptosis; histology rescued | ER-stress & NF-kB suppression; antioxidant |
| Calmaz et al., 2025 | Luteolin | 1 & 5μM (in vitro) | Leydig cells | ↑Viability; ↓ROS/LPO; preserved GSH/enzymes; ↓pro-apoptotic genes | Antioxidant; anti-apoptotic |
| Ara et al., 2021 | Green tea extract | Extract co-admin, PO | Mouse | Improved serum markers & histology (liver/kidney/testis) except highest AA | Polyphenolic antioxidant action |
| Daoudi et al., 2025 | Teucrium polium (aqueous extract) | 400mg/kg PO | Rat | ↑$T_3$/$T_4$, ↑sperm/testosterone; thyroid/testis histology improved | Antioxidant; endocrine restoration |
| Davoudimoghadam et al., 2022 | Vitamin C | 200mg/kg PO | Rat (kidney) | ↓Urea/creatinine/MDA; ↑TAC; renal histology rescued | ROS scavenging; TAC ↑ |
| Edres et al., 2021 | Allicin; Melatonin | Allicin 20mg/kg PO; Melatonin 10 mg/kg (3×/wk) | Rat (brain) | Restored GSH; ↓LPO/8-OHdG; normalized NTs; improved histology | Antioxidant; Nrf2/NF-kB modulation |
| El-Beltagi & Ahmed, 2016 | Quercetin | 50 & 100mg/kg PO | Rat | ↓LPO; ↑GSH/SOD/CAT/GPx; serum markers improved | Phenolic antioxidant |
| Elkomy et al., 2018 | Clove oil (Syzygium aromaticum) | 100 & 200mg/kg PO | Rat | ↓ALT/AST/urea/creatinine; ↑testosterone; ↓MDA; ↑SOD; histology better | Antioxidant/anti-inflam |
| Elsayed et al., 2024 | CoQ10; Rosuvastatin | 10mg/kg each PO | Rat (testis) | ↑Hormones & antioxidants; ↓MDA; ↓caspase-3; morphometry improved (combo best) | Mito-bioenergetics; anti-oxidant/anti-apoptotic |
| El-Shehawi et al., 2022 | Taify pomegranate juice | 1mL $kg^{-1}$ PO | Rat (liver) | Restored antioxidant enzymes; ↓MDA/NO/cytokines; gene shifts toward protection | Nrf2/HO-1 up; anti-inflam/apoptotic |
| Essa et al., 2025 | Ferulic acid (nano-encapsulated in sesame protein isolate) | 20mg/kg PO | Rat | ↓Liver enzymes/MDA/NO/IL-6/TNF-α; ↑GSH/SOD/CAT/GPx; ↓DNA damage; histology better | Antioxidant; anti-inflammatory; anti-genotoxic |
| Famurewa et al., 2024 | Thymoquinone | 5mg/kg PO | Rat (cerebellum) | ↑AChE, ↑SOD/CAT/GPx; ↓MDA; ↓IL-6/TNF-α; Nrf2↑, NF-kB↓; ↓caspase-3/9; histology | Nrf2 activation; NF-kB suppression |
| Farag et al., 2021 | Portulaca oleracea seeds extract | 200/400mg/kg PO | Rat (testis) | ↑Sperm/testosterone; ↓MDA & apoptosis; ↑CYP11A1/17β-HSD | Antioxidant; steroidogenesis support |

| | | | | | |
|---|---|---|---|---|---|
| Gao et al., 2023 | Resveratrol | 50 PO | Mouse (testis) | Restored meiosis markers; ↓γH2AX/p-ATM/p-CHK2; improved sperm indices | Anti-oxidative/anti-DSB signalling |
| Gupta et al., 2025 | Caffeic acid | 10–40mg/kg PO ×3 d (post-AA) | Rat (testis) | Hormones & sperm restored; ↓LPO; ↑GSH/SOD/CAT; ↓DNA damage | ROS scavenging; anti-apoptotic |
| Hasanin et al., 2018 | Vitamin E | 400mg/kg/day PO | Rat (testis) | Preserved seminiferous structure; mitigated ultrastructural damage | Lipid peroxidation inhibition; anti-apoptotic |
| Hong et al., 2021 | Rosmarinic acid | 0, 25, and 50μM pretreatment | BRL-3A | ↓ROS/MDA; ↑GSH/SOD; ↓GRP78/CHOP/IRE1α; ↓p-JNK/ERK/p38; ↓apoptosis | OS & ER-stress inhibition |
| Erdemli et al., 2019 | Vitamin E | 100mg/kg PO × 21 d (gestation) | Rat (offspring testis) | ↑T & antioxidants; ↓MDA; improved histology | ROS scavenging; anti-apoptotic |
| Firouzabadi et al., 2022 | Ascorbic acid | 200mg/kg PO | Rat (ovary) | ↓TOS & BAX/BCL-2 ratio; ↑TAS | Antioxidant & anti-apoptotic |
| Khudair, 2016 | Selenium & Melatonin | Se 0.2 + Mel 10mg/kg PO × 35 d | Rat (metabolic syndrome) | Normalized lipid/glucose & antioxidants | Antioxidant enzyme stimulation (GSH/SOD) |
| Khyoon & Abdulwahid, 2024 | Curcumin | 100mg/kg PO × 40 d | Rat (brain) | ↓MDA; ↑TAC; improved behaviour & histology | Antioxidant; neuroprotective |

**Outcomes and Effect Sizes (for Meta-Analysis Candidates)**

**Primary Outcomes: Oxidative Stress and Reproductive Indices**

Animal studies using rodents have shown that exposure to acrylamide typically raises levels of TBARS or MDA, markers of lipid peroxidation, by 50–200%. However, when antioxidants like vitamin E, zinc, silymarin, morin, or selenium were given along with acrylamide, MDA levels dropped significantly, by 30–60%, compared to groups that only received acrylamide (Teodor et al., 2011; Salman et al., 2020; Sayed et al., 2022; Sozen et al., 2024). Similarly, the activity of antioxidant enzymes (SOD, CAT, GPx, GSH) decreased by 25–70% after acrylamide exposure, compared to control groups. Experimental Characteristics of In Vivo Studies Evaluating Antioxidant Interventions against Acrylamide-Induced Toxicity (Table 6). Giving vitamin E or zinc helped bring these enzyme levels back up, by 40–90%, suggesting a return to almost normal redox balance (El-Beltagi and Ahmed, 2016; Famurewa et al., 2024; Ajibare et al., 2025).

Sperm quality was one of the most easily affected aspects of reproduction. In rats and mice, acrylamide exposure led to a 30–60% decrease in the total number of sperm, a 40–70% decrease in sperm movement, and a 50–100% increase in abnormally shaped sperm (Duan et al., 2015; ALKarim et al., 2015; Zhang et al., 2022b). Antioxidants in the testicles, like vitamin E, morin, and green-tea catechins, improved these measures by 25–55% compared to groups exposed to the toxic substance alone (Kucukler et al., 2020). After repeated exposure to acrylamide, testosterone levels fell by 35–60%, while luteinizing hormone and follicle-stimulating hormone showed inconsistent decreases of 15–40%, indicating problems with Leydig cells (Ahmed et al., 2022; Mokhlis et al., 2023).

When germ cells were exposed to AA, their mitochondrial membrane potential (Δψm) dropped significantly, usually by 40–60%. This decrease was associated with ATP depletion and the release of cytochrome c (Omidi et al., 2020; Gao et al., 2021). However, when melatonin, NAC, or morin were given alongside AA, Δψm was restored by 30–50%, suggesting some recovery of cellular energy production (Kucukler et al., 2020; Edres et al., 2021; Ozturk et al., 2023). Acrylamide exposure also caused a 2- to 3-fold increase in 8-hydroxy-2′-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, especially in sperm-producing cells (Kucukler et al., 2020; Zhang et al., 2023).

After exposure to AA, caspase-3 activity, a key indicator of apoptosis, increased by 60–120%. The Bax/Bcl-2 ratio also increased by 1.5-2.5 times, which supports the activation of the mitochondrial apoptotic pathway (El-Shehawi et al., 2022; Firouzabadi et al., 2022). However, antioxidants like melatonin, morin, or silymarin were able to reverse these changes by reducing caspase-3 expression and restoring the Bax/Bcl-2 balance to normal (Kandemir et al., 2020; Dag et al., 2025). AA exposure also led to a 1.8–3-fold increase in NF-kB activation and inflammatory cytokines (TNF-α, IL-6). Antioxidant treatments reduced these increases by 40–70%, showing their ability to fight inflammation (Pan et al., 2018; Amirshahrokhi, 2021).

When examining tissue samples under a microscope, the seminiferous tubules showed damage that worsened with increasing doses. Acrylamide led to a 30–60% decrease in the diameter of these tubules and the height of their germinal epithelium. Common problems included seminiferous atrophy, loss of germ cells, and the formation of vacuoles (Hasanin et al., 2018; Gupta et al., 2025). However, protective substances like vitamin E, selenium, zinc, and silymarin improved the structural health of these tubules by 40–70%, helping to restore their normal shape and the layers of cells involved in sperm production (Alturki et al., 2022; Siddiqa et al., 2025).

**Effect Size Estimates for Meta-Analysis**

Early combined results from relevant studies on rodents indicate that the standardized mean differences (SMDs) for MDA levels were between +1.2 and +2.5 when comparing groups exposed to AA with control groups. For SOD, CAT, and GPx, the SMDs were between 1.0 and 2.0, suggesting large effects (Ibaokurgil et al., 2023; Uslu et al., 2025). Sperm count and movement usually showed SMDs between 1.1 and 1.8, and testosterone levels decreased with an SMD around 1.3 (Gupta et al., 2025; Moradi et al., 2025). It's likely that the variation ($I^2$) between studies will be over 60%, due to differences in study design, types of antioxidants used, and experimental setups (Ebrahimi et al., 2024).

**Table 6:** Experimental Characteristics of In Vivo Studies Evaluating Antioxidant Interventions Against Acrylamide-Induced Toxicity

| Study (Year) | Species/Strain | Age | Acrylamide Dose (mg/kg/day) | Route of Exposure | Duration (Days) | Antioxidant Type/Form | Outcome Summary |
|---|---|---|---|---|---|---|---|
| Kaçar et al., 2018 | Wistar rat | Adult | 40 | Intraperitoneal | 10 | L-Cysteine (pure compound) | Partial protection |
| Farag at et al., 2021 | Wistar rat | Adult | 10 | Oral | 60 | Portulaca oleracea seed extract (plant extract) | Significant protection |
| Yildirim et al., 2023 | Wistar rat | Adult | 30 | Oral | 10 | Selenium (elemental compound) | Full protection |
| Radad et al., 2020 | Sprague-Dawley rat | Adult | 30 | Oral | 10 | Minocycline (drug antioxidant) | Marked protection |
| Ahmed et al., 2022 | Wistar rat | Adult | 25 | Oral | 30 | Earthworm methanolic extract | Moderate protection |
| Abd-Elghaffar et al., 2015 | Wistar rat | Adult | 20 | Oral | 30 | Garlic oil (natural extract) | Significant protection |

**Moderators of Heterogeneity**

The effectiveness of antioxidants and measures of oxidative stress can vary because of several important factors. These include the type of animal used (such as different breeds of rats or mice), the animal's age (young versus adult), the amount of antioxidant given (from 2 to 50 mg per kg of body weight per day), how it's given (by mouth or injection), and how long it's given for (a short period like a week, or a longer period like a month or two). The amount and type of antioxidant used (whether it's a pure substance or an extract, and whether it's in a nano-formulation or a conventional one) also have a big impact on the results. Generally, larger doses of antioxidants led to better recovery from oxidative stress, but they also created more variation because of differences in how well the body could use them and when the treatment was given (Kaçar et al., 2018; Radad et al., 2020; Farag et al., 2021).

**Meta-Analysis Suitability and Data Quality Considerations**

Studies that give us the average (mean) and standard deviation (SD) for oxidative stress markers (like MDA, SOD, CAT, GPx, GSH) and reproductive measures (sperm count, motility, testosterone) are the best for combining data in a quantitative way. Other markers, such as Δψm, 8-OHdG, and caspase-3, can tell us about the mechanisms involved, but they aren't reported as consistently and might be better used to describe the studies in a general way instead of being combined statistically. Funnel-plot asymmetry and Egger's regression should be applied to evaluate publication bias, while subgroup analysis across exposure duration, antioxidant class, and dosage will refine pooled effect interpretation.

**Risk of Bias in Animal Studies**

Assessment of experimental rigor using SYRCLE's Risk of Bias tool revealed several recurrent methodological limitations among included in vivo studies. Random sequence generation was described in less than 40% of studies, while allocation concealment was almost universally unreported (Zhang et al., 2019; Wilson et al., 2023). Lack of blinding of investigators or outcome assessors was another major source of performance and detection bias, as histopathological and biochemical endpoints were often measured by the same research teams aware of treatment allocation (Hartung et al., 2025). Selective outcome reporting was noted in several publications where negative or statistically non-significant findings for certain parameters (e.g., hormonal assays or minor oxidative biomarkers) were omitted from result tables (Sengul et al., 2023; Yildirim et al., 2024).

The attrition bias remained generally low, as most animal studies reported complete datasets and survival rates. However, other biases such as absence of random cage allocation, unclear housing conditions, and lack of power calculations contributed to moderate risk classifications overall. Only a minority of studies employed explicit compliance with OECD or ARRIVE guidelines. Collectively, the methodological quality of animal studies was graded as moderate, with most rated as having some concerns in 3–4 of the 10 SYRCLE domains.

**Risk of Bias in In Vitro and Cellular Studies**

In vitro investigations primarily sperm or Leydig/Sertoli cell models were generally limited by absence of replication across independent laboratories and lack of predefined sample-size justification. Although assay reproducibility and quantitative controls were adequate, experimenter blinding and randomization of exposure concentrations were seldom described (Eisenbrand, 2020; Dos Santos et al., 2023). Reporting completeness was variable, especially regarding culture conditions and antioxidant treatment timing, yielding an overall moderate-to-high risk of bias classification for cell-based datasets.

**Risk of Bias in Human Observational Evidence**

The limited human data available, mostly small cross-sectional or occupational biomonitoring studies were assessed using the ROBINS-I framework and consistently demonstrated high risk of confounding and selection bias (Filippini et al., 2022). Key confounders such as diet, smoking status, age, and co-exposure to other toxicants were not always controlled. Additionally, exposure misclassification (dietary estimation rather than measured biomarkers) and outcome assessment heterogeneity (variable semen-analysis protocols) further reduced internal validity. None of the studies included longitudinal follow-up or repeated exposure measurements, resulting in overall low methodological robustness and very low certainty under GRADE criteria.

**Certainty of Evidence (GRADE Evaluation)**

Using the GRADE approach, confidence in the body of evidence was rated as moderate to low for animal studies and very low for human observational data. The main downgrading factors were risk of bias, inconsistency, and indirectness of evidence.

Animal Studies: Certainty was graded moderate for oxidative stress and sperm outcomes due to consistent directionality across multiple species and antioxidant classes. Evidence was downgraded for imprecision and incomplete reporting of randomization. Certain parameters

**Table 7:** Risk of Bias Summary (SYRCLE/ROBINS-I)

| Study | Randomization | Allocation Concealment | Blinding | Attrition Bias | Reporting Bias | Overall Risk |
|---|---|---|---|---|---|---|
| Ajibare et al., 2024 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| Gür et al., 2023 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Anvari et al., 2020 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Trigg et al., 2021 | Low | Unclear | Unclear | Low | Low | Moderate |
| Ligina et al., 2022 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Üremiş et al., 2024b | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Sedik et al., 2025 | Low | Unclear | Unclear | Low | Low | Moderate |
| Abd Al Haleem et al., 2022 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Abdel-Daim et al., 2020 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Abd-Elghaffar et al., 2015 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Abd-Elsalam et al., 2021 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Abduljalil et al., 2024 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Ahmed et al., 2022 | Low | Unclear | Unclear | Low | Low | Moderate |
| ALKarim et al., 2015 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Alturki et al., 2022 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Amirshahrokhi, 2021 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| Atasever et al., 2025 | Low | Unclear | Unclear | Low | Low | Moderate |
| Banc et al., 2022 | Low | Unclear | Unclear | Low | Low | Moderate |
| Baraka et al., 2024 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Bhuiyan et al., 2023 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| Cakmak et al., 2025 | Low | Unclear | Unclear | Low | Low | Moderate |
| Daoudi et al., 2025 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| Davoudimoghadam et al., 2022 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| Dos Santos et al., 2023 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| Duan et al., 2015 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Edres et al., 2021 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| El-Beltagi & Ahmed, 2016 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Elkomy et al., 2018 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| Elsayed et al., 2024 | Low | Unclear | Unclear | Low | Low | Moderate |
| Erdemli et al., 2019 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Essa et al., 2025 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| Famurewa et al., 2024 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| Farag et al., 2021 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| Firouzabadi et al., 2022 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| Gao et al., 2021 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Gao et al., 2023 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| Gupta et al., 2025 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| Hasanin et al., 2018 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Mostafa-Hedeab et al., 2023 | Low | Unclear | Low | Low | Low | Low |
| Zhang et al., 2022 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| Yang et al., 2021 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| El-Shehawi et al., 2022 | Low | Unclear | Unclear | Low | Some concerns | Moderate |
| Khudair, 2016 | Unclear | Unclear | Unclear | Low | Some concerns | Moderate |
| Khyoon & Abdulwahid, 2024 | Low | Unclear | Unclear | Low | Some concerns | Moderate |

(e.g., MDA, SOD, testosterone) qualified for upgrading because of large and consistent effect magnitudes (>1 SD change) and clear dose–response gradients (Kucukler et al., 2020; Ajibare et al., 2024).

Cellular Studies: Certainty was rated low, constrained by limited replication and lack of standardized methodologies for oxidative markers and apoptotic assays. However, reproducible concentration-dependent responses for ROS and mitochondrial depolarization partially justified upgrading for consistency (Gupta et al., 2023; Yildirim et al., 2023).

Human Evidence: Certainty was graded very low due to cross-sectional design, small sample sizes, exposure misclassification, and residual confounding (Kashani et al., 2021; Yildirim et al., 2023). No direct prospective or interventional data currently exist linking Hb adducts or dietary AA exposure to semen quality or hormonal profiles. Risk of Bias summary presented in Table 7.

## DISCUSSION

This systematic review of literature indicates strongly that oxidative stress plays a major role in the mechanism of being sickened by acrylamide (AA) in the testicles and reproductive system. Research with different rodent models revealed that exposure to AA resulted in a substantial increase in lipid peroxidation markers (such as MDA and TBARS) and a substantial decrease in antioxidant enzymes (such as SOD, CAT, GPx and GSH) (Banc et al., 2022; Onur et al., 2024). The presence of this imbalance between oxidants and antioxidants was directly linked to the decreased count of sperm, their movement and shape, and a lowered production of testosterone, which proved that there is a relationship between the presence of an oxidative damage and the development of reproductive issues (Seify et al., 2024; Yildirim et al., 2024). Antioxidant treatments have continuously corrected oxidative stress markers and enhanced reproduction. Such substances as vitamin E, zinc, selenium, and morin restored enzyme activity to approximately normal levels, decreased lipid peroxidation, and improved the quality of sperms, and maintained evidence that addressing oxidative stress can effectively reduce testicular damage induced by AA (Kucukler et al., 2020; Yildirim et al., 2024; Üremiş et al., 2024). Experiments on the mechanistic aspects of the action of acrylamide have implicated two main regions at

the molecular scale: destabilization of the Nrf2/Keap1 antioxidant system and mitochondrial damage. Acrylonitrile prevents Nrf2 to migrate to a cell nucleus and decrease the expression of antioxidant genes (HO-1 and NQO1), which suppresses the cell defense ability (Zhao et al., 2017; Ajibare et al., 2024). Simultaneously, it interferes with the mitochondrial membrane, causing the decrease in the ATP level and the extraction of cytochrome c, which triggers an occurrence of a programmed cell death. The mechanisms of this process are the imbalance of Bax/Bcl-2 proteins and the activation of caspase-3 (Deng et al., 2022; Chen et al., 2023). Cumulative effects of these problems are over generation of reactive oxygen species, loss of energy and germ cell death (Naseer et al., 2025). Resources capable of revitalizing Nrf2 and reinforcing mitochondrial membranes, such as melatonin, zinc and selenium have the greatest potential to reverse such effects. This implies that the inhibition of these pathways might be a helpful solution in alleviating the reproductive toxicity of acrylate in reducing acrylonitrile (Ajibare et al., 2025; Khan et al., 2024). Although the bulk of the evidence is provided by those studies that are conducted on animals in labs, how these findings apply to the human biology is quite convincing. Measured studies of people do indicate that even non-smokers are found to have some amount of certain compounds (HbAA, HbGA) in their blood and that the level of smokers and individuals that work in certain industries is 3 to 4 times higher (Duke et al., 2018; Liu et al., 2021). We suppose that the daily amount of these received through diet is about 0.4 to 1.9 micrograms per kilogram of body weight, which is very low compared to those in animal experiments (2 to 50 milligrams per kilogram of body weight per day). Nevertheless, long-term use and how our bodies metabolize these substances with the help of an enzyme known as CYP2E1 might accumulate to the point of influencing our health (Pietropaoli et al., 2022). When we generalize such findings to individuals, it implies that individuals who consume large amounts of foods with these compounds, lack sufficient antioxidants in their diets or have other factors that put their bodies under stress (such as smoking, diabetes, or obesity) may be more prone to develop problems regarding their reproduction. Due to this, basic and cheap items such as consuming more foods that are rich in vitamin E, selenium and polyphenols may aid in maintaining the balance of the body. But we should have more direct research in humans and we should be cautious in extrapolating findings in animal research to humans, particularly as regards the extent to which one species is similar to another, how quickly their systems react to things and their ability to absorb antioxidants. Medically and in the perspective of the public health, the exposure to these compounds ought to be a risk factor which we can modify whenever examining the etiology of infertility. Men with unexplainable infertility, evidence of higher stress levels in their bodies, or having been exposed to such compounds in the workplace may need guidance on eating low amounts of the compounds (frying and processed foods) and taking antioxidant supplements upon request. For smokers, cutting back on tobacco could greatly reduce both their exposure to these compounds and the stress on their bodies. Also, taking antioxidants that have been shown to help, such as vitamin E, zinc, selenium and melatonin, might improve sperm quality, as shown in studies on toxins and fertility (Chu et al., 2020; Sahu et al., 2020). Since stress in the body is a common factor in many different causes of infertility, managing this imbalance could be a general way to help, even in cases not specifically related to these compounds.

Despite the strong empirical coherence, there are several limitations that limit the degree of certainty with which conclusions may be drawn from these results. Positive publication bias for antioxidant efficacy is likely inflating apparent efficacy; few studies have reported null or negative results. Heterogeneity of animal species, doses, exposure routes and antioxidant supplementation preclude the pooling through meta-analysis. Antioxidant therapies differ in dose of the treatment (10–200mg/kg), duration (7–90 days) and timing prophylactic vs. therapeutic, limiting comparison between studies. Moderate risk for bias is also represented by absence of RCTs or blinded assessments in animal studies (Filippini et al., 2022; Mattosinhos et al., 2022). Data on humans are limited and cross-sectional; therefore, causal inference cannot be made. Additionally, a lack of uniformity in reporting and consistency within hormonal assays, histological scoring and redox parameters cause methodological heterogeneity.

Although the use of antioxidant supplements has great therapeutic possibilities, overdose also has potential risks. Vitamin E at high doses may interfere with vitamin K-dependent coagulation, selenium at excess levels might lead to selenosis, and over-supplementation of zinc can have an adverse effect on copper metabolism (Vinceti et al., 2017; Wahab et al., 2020; Abrol et al., 2023). Certain antioxidants show hormesis, in that low doses are protective from the same compound, while higher doses paradoxically potentiate oxidative damage or inhibit endogenous antioxidant signaling (Nitti et al., 2022). Thus, it is crucial to determine ideal dosage regimes and strive for antioxidant efficacy while also promoting a physiological adaptation. Furthermore, drug–nutrient and nutrient–nutrient interactions should be explored in the design of human interventional trials.

Standardized dosing regimens, blinded randomized animal protocols and dose-response (both acute effects and withdrawal-recovery) designs should be used in future studies to ascertain whether oxidative- and endocrine-related changes can be reversed. From a translational angle, prospective human cohorts combining Hb-adduct biomonitoring, semen samples, hormonal profiling and dietary antioxidant monitoring seem warranted. And lastly, the development of novel delivery systems as nano-antioxidants or dual combination therapies could promote testis-targeting delivery and increase bioavailability with reduced systemic toxicity (Alturki et al., 2022; Dahran et al., 2023; El-Kossi et al., 2023).

**Future Directions**

**1) Standardized antioxidant trials in AA-exposed men (workers/smokers)**

Design multicenter, randomized, placebo-controlled trials targeting occupations with documented acrylamide

exposure and smokers with elevated Hb adducts; stratify by baseline HbAA/HbGA to enrich for exposure (Obón-Santacana et al., 2017; Vallejo, 2024). Test dose–response and combinations of evidence-supported agents (e.g., vitamin E, selenium, zinc, melatonin) with harmonized regimens and outcome windows drawn from preclinical efficacy (Khudair, 2016; Ajibare et al., 2024; Yildirim et al., 2024; Dag et al., 2025; Ebaid et al., 2025). Primary endpoints: semen quality (count/motility/morphology), oxidative-stress markers (MDA/TBARS; SOD/CAT/GPx/GSH) and hormones; secondary endpoints: mitochondrial function (Δψm in sperm), apoptosis indices (caspase-3; Bax/Bcl-2) and histopathology where applicable (Seify et al., 2024; Deng et al., 2022).

**2) Nrf2-targeted and Mitochondria-directed Therapeutics**

Advance agents that restore Nrf2/HO-1 signaling and stabilize mitochondrial function—mechanistic nodes repeatedly implicated in benefit (Abd-Elsalam et al., 2021; Rotimi et al., 2022). Prioritize candidates with dual actions (e.g., melatonin; zinc/selenium) and explore mitochondria-targeted antioxidants (e.g., MitoQ) in AA models to validate Δψm rescue, ATP restoration and apoptosis reduction before first-in-human studies (Kucukler et al., 2020; Yildirim et al., 2024).

**3) Integrated Biomarker Panels for Translational Monitoring**

Implement composite panels combining Hb adducts (HbAA/HbGA) for internal dose, semen redox markers (MDA/TAC; SOD/CAT/GPx/GSH) and DNA oxidation (8-OHdG) to link exposure → mechanism → function across time (Mannucci et al., 2022; Albiach-Delgado et al., 2022; Mottola et al., 2024). Use repeated measures (baseline, mid-intervention, post-intervention) to capture temporality and to support mediation analyses.

**4) Delivery science and pharmacokinetics**

Develop nanoemulsions/liposomal systems for low-bioavailability polyphenols and micronutrients; characterize PK/PD, tissue distribution and reproductive safety in AA models prior to translation (Bučević Popović et al., 2024; Ajibare et al., 2024). Benchmark against conventional formulations on identical AA regimens to quantify absolute gains in testicular targeting and effect size (Ahmed-Farid et al., 2017; Yildirim et al., 2024).

**5) Systems toxicology to map AA–antioxidant networks**

Apply multi-omics (metabolomics, proteomics, transcriptomics, epigenomics) on testes and sperm to reconstruct redox, inflammatory and steroidogenic pathways perturbed by AA and rescued by specific antioxidants (Signorini et al., 2024; Fang et al., 2025). Integrate pathway analysis with mitochondrial assays and Nrf2-target engagement to identify predictive biosignatures for response.

**6) Male Preconception Care Frameworks**

Embed AA mitigation into preconception clinics: brief interventions on diet (lower AA foods), smoking cessation to reduce adduct burdenand targeted antioxidant support guided by baseline redox profiling (Kashani et al., 2021; Šebeková et al., 2025). Develop pragmatic algorithms that trigger supplementation when Hb adducts or semen oxidative markers exceed thresholds validated in trials (Obón-Santacana et al., 2016; Fallah et al., 2018; Vryonidis et al., 2024).

**7) Study Design and Reporting Standards**

Adopt ARRIVE-compliant randomized animal studies with blinding and power calculations; in human studies, use prespecified statistical plans controlling for diet, smoking, BMI and co-exposures; report full biomarker panels and raw summary data to reduce selective reporting and enable meta-analysis (Percie du Sert et al., 2020; Kucukler et al., 2020).

**8) Fundable Milestones**

Year 1–2: finalize panels, complete nano-PK/PD and dose-finding in AA rodent models; Year 2–4: pilot RCTs in smokers/workers with biomarker-anchored endpoints; Year 4–5: scale multicenter trials with combination arms, validate omics-based response signatures and publish clinical guidance (Kaltsas, 2025; Kong et al., 2025).

**Conclusion**

This systematic review concludes that acrylamide (AA) exposure induces significant oxidative stress-mediated testicular toxicity through mechanisms involving excessive generation of reactive oxygen species (ROS), mitochondrial dysfunction, apoptosis, and hormonal imbalance. Experimental evidence consistently demonstrated elevated lipid peroxidation markers such as TBARS/MDA, along with reductions in antioxidant enzymes including SOD, CAT, GPx and GSH. Molecular data highlighted AA-induced suppression of the Nrf2/HO-1 pathway, disruption of the Bax/Bcl-2 ratio and activation of caspase-3, collectively leading to germ cell apoptosis and decreased testosterone synthesis. Antioxidant interventions, including vitamins (C and E), thiols (N-acetylcysteine), polyphenols (resveratrol, quercetin, curcumin), melatonin and trace elements (selenium, zinc), showed protective effects by enhancing endogenous antioxidant defenses, restoring hormonal levels and improving sperm quality. Despite moderate-to-low certainty of evidence due to study heterogeneity and limited human data, the findings strongly support oxidative stress as the core mechanism of AA-induced reproductive toxicity and reinforce the potential of antioxidant therapy as an effective prophylactic and therapeutic approach for mitigating male reproductive dysfunctions associated with acrylamide exposure.

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