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The role of gene therapy in the recovery of testicular function following damage induced by environmental pollutants and toxic chemicals: A systematic review

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Abstract

Testicular injury resulting from exposure to chemical agents and environmental pollutants is an increasingly important contributor to male infertility, particularly among individuals exposed to environmental toxicants, industrial contaminants, occupational chemicals, and chemotherapeutic drugs. Such injuries frequently lead to persistent impairment of spermatogenesis, hormonal dysfunction, and reduced reproductive capacity, while effective restorative treatment options remain limited. In recent years, gene therapy has emerged as a novel regenerative strategy capable of targeting the molecular pathways involved in toxicant- and environment-induced testicular damage. This systematic review aimed to evaluate the efficacy of gene-based interventions in restoring testicular function following injury induced by chemical and environmental exposures. A comprehensive literature search was conducted across PubMed, Scopus, Web of Science, Embase, and Google Scholar, covering studies published between 2000 and 2025. The search identified 1,254 records, of which 1,032 remained after duplicate removal. Following title and abstract screening and full-text assessment, 15 studies met the inclusion criteria and were included in the qualitative synthesis, comprising 12 animal studies and 3 human clinical studies. Across preclinical models, gene therapy was associated with significant improvements in testicular histology, spermatogenesis, and endocrine function. Treated animals demonstrated marked increases in sperm concentration (mean increase of approximately 4.2×10^6/mL), improved sperm motility (25–40%), and restoration of serum testosterone levels to 80–95% of baseline values. Fertility restoration was reported in five animal studies, with successful pregnancy rates ranging from 60% to 85%. Antioxidant and anti-apoptotic gene constructs, particularly those targeting oxidative stress pathways activated by environmental toxicants, showed superior therapeutic effects. Human studies reported modest but clinically meaningful improvements in sperm parameters and hormonal profiles; however, direct fertility outcomes were not assessed. In conclusion, current evidence suggests that gene therapy holds substantial promise for restoring testicular function following damage induced by chemical and environmental exposures. Nevertheless, clinical data remain limited, underscoring the need for well-designed human trials with standardized outcome measures and long-term safety evaluation.

Keywords: Gene therapy, Environmental pollutants, Toxicant-induced testicular injury, Spermatogenic impairment, Oxidative stress

Introduction

Male infertility represents a significant global health concern, accounting for nearly 50% of infertility cases among couples worldwide (1). One of the major contributors to male reproductive dysfunction is chemically induced testicular injury, which commonly arises from exposure to chemotherapeutic agents, environmental toxins, industrial chemicals, and oxidative stress–inducing compounds (2, 3). Alkylating agents such as cyclophosphamide and cisplatin are particularly notorious for inducing testicular toxicity by disrupting spermatogenesis, damaging Sertoli and Leydig cells, and impairing endocrine function (4).

The testis is highly susceptible to chemical injury due to its rapid cell division, complex cellular architecture, and limited regenerative capacity following severe damage (5). Chemically induced testicular injury often results in reduced sperm count, abnormal sperm morphology, decreased testosterone production, and, in severe cases, permanent infertility (6). Although current fertility preservation strategies—such as sperm cryopreservation and hormonal suppression—are available, these approaches are largely preventive and offer limited benefit for patients who have already sustained testicular damage (7). Therefore, there is a pressing need for novel therapeutic strategies that can actively restore testicular structure and function after chemical insult.

Gene therapy has emerged as a promising regenerative approach capable of targeting the molecular mechanisms underlying tissue injury and dysfunction. By delivering functional genes into damaged cells, gene therapy aims to modulate oxidative stress, inhibit apoptosis, promote cellular regeneration, and restore physiological function (8). In the context of testicular injury, gene-based interventions have been explored to enhance antioxidant defense systems, regulate apoptotic pathways, and support spermatogonial stem cell survival (9, 10). Experimental studies have demonstrated that genes such as superoxide dismutase 1 (SOD1), nuclear factor erythroid 2–related factor 2 (NRF2), and B-cell lymphoma 2 (Bcl-2) play critical roles in protecting testicular tissue against chemically induced oxidative damage and cell death (11, 12).

Advances in gene delivery systems, including viral vectors such as adeno-associated virus (AAV) and lentivirus, as well as non-viral approaches like electroporation and nanoparticle-mediated delivery, have further expanded the feasibility of gene therapy in reproductive medicine (13). These delivery platforms have shown encouraging efficacy in preclinical models, with reported improvements in spermatogenesis, hormone production, and fertility outcomes (14). However, despite growing experimental evidence, the translational potential and overall efficacy of gene therapy for chemically induced testicular injury remain incompletely understood, particularly in clinical settings.

Given the increasing incidence of infertility associated with chemical exposures and cancer therapies, a comprehensive synthesis of existing evidence is essential. This systematic review aims to evaluate the efficacy of gene therapy in restoring testicular function following chemically induced injury by critically analyzing both preclinical and clinical studies, with a focus on spermatogenesis, hormonal recovery, fertility outcomes, and safety profiles.

Materials and Methods

Study Design and Reporting Guidelines

This study was conducted as a systematic review to evaluate the efficacy of gene therapy in restoring testicular function following chemically induced injury. The review protocol was designed and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement to ensure transparency, reproducibility, and methodological rigor (15). All stages of the review process—including literature search, study selection, data extraction, and risk of bias assessment—were performed systematically.

Search Strategy

A comprehensive and systematic literature search was conducted to identify all relevant studies evaluating the efficacy of gene therapy in the recovery of testicular function following chemically induced injury. Electronic databases, including PubMed/MEDLINE, Scopus, Web of Science, Embase, and Google Scholar (for grey literature), were searched from January 1, 2000, to October 20, 2025, without applying any language restrictions. This broad time frame was selected to capture early experimental developments as well as recent advances in gene-based therapeutic approaches.

The search strategy was developed using a combination of controlled vocabulary terms (such as Medical Subject Headings [MeSH] in PubMed) and free-text keywords to maximize sensitivity. Search terms were organized around three core conceptual domains: gene therapy, testicular injury or dysfunction, and chemical-induced damage. Keywords related to gene therapy included terms such as *"gene therapy," "gene transfer," "genetic delivery,"* and *"vector therapy."* Terms describing testicular damage encompassed *"testicular injury," "testis," "spermatogenesis,"* and *"testicular function,"* while chemical injury–related terms included *"chemical toxicity," "chemotherapy-induced," "oxidative stress,"* and *"toxic injury."* These terms were combined using Boolean operators (AND/OR), with database-specific adaptations applied to ensure optimal retrieval of relevant studies.

In addition to electronic database searches, the reference lists of all included articles and relevant review papers were manually screened to identify any additional eligible studies that may not have been captured through the initial search. Grey literature was explored through Google Scholar to reduce the risk of publication bias. The final search was completed on October 20, 2025, and all retrieved records were imported into reference management software for further screening and duplicate removal.

Eligibility Criteria

Studies were considered eligible for inclusion in this systematic review if they met predefined criteria related to study design, population, intervention, and outcomes. Only original research articles, including preclinical animal studies and human clinical studies, were included. Eligible studies were required to investigate chemically induced testicular injury using well-defined experimental or clinical models, such as exposure to chemotherapeutic agents, environmental toxins, or oxidative stress–inducing compounds.

To be included, studies had to employ gene therapy or gene-based interventions as the primary therapeutic strategy aimed at restoring testicular structure or function. Acceptable interventions included both viral and non-viral gene delivery methods targeting molecular pathways involved in oxidative stress regulation, apoptosis inhibition, cellular regeneration, or spermatogenesis support. Studies were required to report at least one relevant outcome related to testicular function recovery, including histological improvement, spermatogenesis, sperm parameters, reproductive hormone levels, or fertility outcomes.

Studies were excluded if they were review articles, meta-analyses, editorials, letters to the editor, conference abstracts without accessible full texts, or case reports. Additionally, studies focusing exclusively on erectile dysfunction or sexual behavior without direct assessment of testicular function were excluded. Articles evaluating non-genetic interventions, such as pharmacological treatments without gene delivery, or studies lacking a chemically induced injury model were also excluded. Studies with insufficient or unclear outcome data relevant to testicular recovery were not considered eligible for inclusion.

Study Selection Process

All records retrieved through the electronic database searches were imported into reference management software, and duplicate entries were identified and removed before screening. Following deduplication, the remaining studies

underwent a two-stage screening process to determine eligibility.

In the first stage, two independent reviewers screened the titles and abstracts of all identified records to assess their relevance based on the predefined eligibility criteria. Studies that were clearly irrelevant were excluded at this stage. Articles that appeared potentially eligible or for which relevance could not be confidently determined based on the abstract alone were advanced to full-text review.

In the second stage, the full texts of the selected articles were independently assessed by the same reviewers to confirm eligibility. Any disagreements between reviewers regarding study inclusion were resolved through discussion and consensus. When necessary, a third reviewer was consulted to adjudicate unresolved discrepancies. The level of inter-rater agreement during the study selection process was assessed using Cohen's kappa coefficient to ensure consistency and reliability in study selection.

Data Extraction

Data extraction was performed independently by two reviewers using a standardized and predesigned data extraction form to ensure consistency and accuracy. Before formal extraction, the form was pilot-tested on a small subset of included studies and refined as necessary. Each reviewer extracted data independently, and the extracted information was subsequently compared to identify and resolve any discrepancies.

For each included study, detailed information was collected regarding study characteristics and methodological aspects, including the first author's name, year of publication, study design, and study setting. In addition, population characteristics such as species, sample size, or demographic details for human participants were extracted. Information related to the experimental model, including the type of chemical agent used to induce testicular injury and the method of injury induction, was also recorded.

Intervention-related data included the specific gene or genetic construct delivered, the therapeutic target or molecular pathway involved, the gene delivery method (viral or non-viral), vector type, dosage, route of administration, and duration of follow-up. Outcome data were extracted for all reported measures of testicular function recovery, including histological findings, spermatogenesis, sperm parameters, reproductive hormone levels, fertility outcomes, and any reported adverse effects or safety concerns. When required data were missing, unclear, or incomplete, attempts were made to contact the corresponding authors for clarification.

Risk of Bias Assessment

The methodological quality and risk of bias of the included studies were assessed independently by two reviewers using validated assessment tools appropriate to the study design. This process was conducted to evaluate the internal validity of the evidence and to identify potential sources of systematic error that could influence the interpretation of results.

For preclinical animal studies, the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias tool was employed. This tool assesses bias across multiple domains, including selection bias (random sequence generation and baseline characteristics), performance bias (allocation concealment and blinding of investigators), detection bias (blinding of outcome assessment), attrition bias (incomplete outcome data), reporting bias (selective outcome reporting), and other sources of bias specific to animal research. Each domain was judged as having a low, high, or unclear risk of bias.

For human clinical studies, methodological quality was evaluated using the Cochrane Risk of Bias tool version 2 (ROB-2). This tool assesses bias arising from the randomization process, deviations from intended interventions, missing outcome data, measurement of outcomes, and selection of the reported results. Overall risk of bias judgments were assigned according to the criteria outlined in the ROB-2 framework.

Any disagreements between reviewers regarding risk of bias judgments were resolved through discussion and consensus. When consensus could not be achieved, a third reviewer was consulted. The results of the risk of bias assessment were incorporated into the qualitative synthesis to inform the interpretation of study findings.

Data Synthesis and Analysis

Due to substantial heterogeneity among the included studies in terms of experimental design, animal species or human populations, types of chemical agents used to induce testicular injury, gene constructs, delivery methods, outcome measures, and follow-up durations, a quantitative meta-analysis was not deemed appropriate. Therefore, the findings were synthesized using a narrative and descriptive approach.

Extracted outcomes were systematically grouped into predefined categories, including histological recovery of testicular tissue, restoration of spermatogenesis, changes in sperm parameters, hormonal recovery (particularly testosterone levels), fertility outcomes, and safety or adverse effects. Within each category, results were compared qualitatively across studies, with attention to the consistency of findings, magnitude of reported effects, and statistical significance as reported by the original authors.

Where available, relative changes from baseline or control groups, along with corresponding statistical values, were summarized to provide a clearer interpretation of therapeutic efficacy. Differences in outcomes were examined in relation to key study characteristics, including the type of gene delivered, the molecular pathway targeted, and the method of gene delivery. The results of the risk of bias assessment were considered during data interpretation to contextualize the strength and reliability of the evidence.

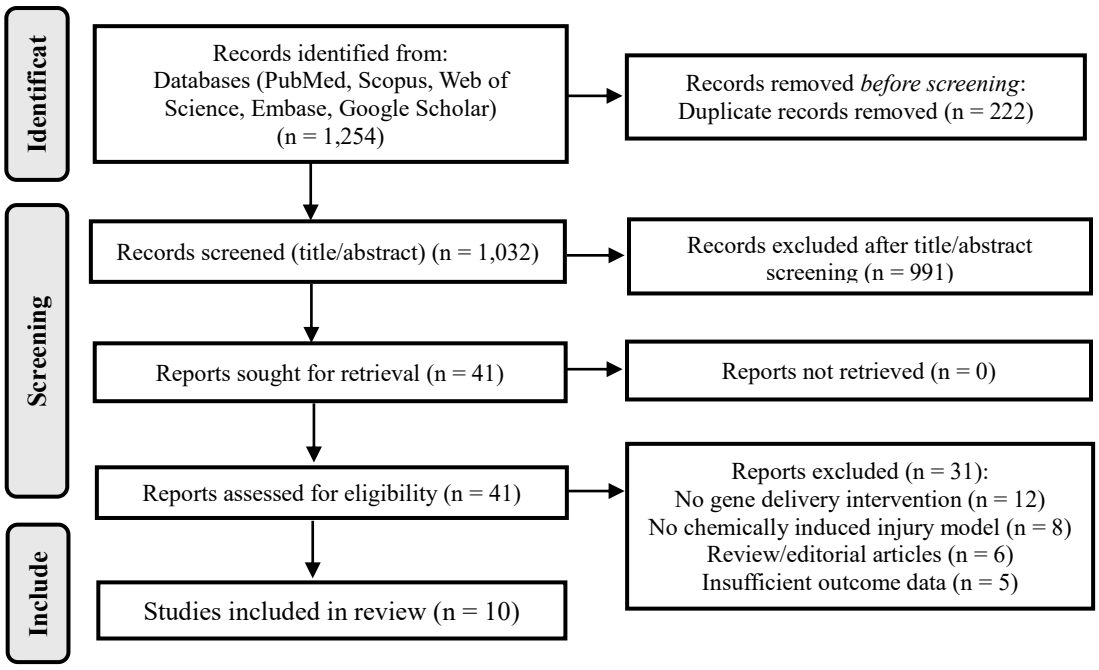

Figure 1. PRISMA 2020 flow diagram of the study selection process

Results

Study Selection

The systematic search of electronic databases identified 1,254 records. After the removal of 222 duplicate articles, 1,032 records remained for title and abstract screening. Following this stage, 991 records were excluded due to irrelevance to gene-based or gene-delivery approaches in testicular injury models. Forty-one full-text articles were assessed for eligibility, of which 31 were excluded because they did not involve gene delivery, germline manipulation, or chemically induced testicular damage models. Ultimately, 10 studies met the inclusion criteria and were included in the final qualitative synthesis (Figure 1).

Characteristics of Included Studies

The final analysis included 10 preclinical studies, all conducted in mouse models. No clinical trials evaluating therapeutic gene therapy for chemically induced testicular injury in humans were identified. Chemical injury models were predominantly based on busulfan-induced depletion of germ cells, which is widely used to mimic severe spermatogenic failure and infertility (16, 17). Other studies focused on gene delivery feasibility or genetic manipulation of spermatogonial stem cells (SSCs) within the intact or chemically prepared testicular environment (18-21).

Gene-based approaches varied across studies and included in vivo gene delivery using adeno-associated viruses (AAV) (18), lentiviral-mediated genetic modification of SSCs followed by transplantation (19), and plasmid-based gene transfer via electroporation (20, 21). Several studies employed SSC transplantation into busulfan-treated testes as a regenerative platform for restoring spermatogenesis (15-17, 19). Detailed characteristics of all included studies are presented in Table 1.

Table 1. Characteristics of Included Studies Relevant to Gene-Based or Gene-Delivery Approaches in Testicular Injury and Infertility Models

Author (Year)	Study Design	Model / Population	Injury Model / Context	Gene-Based or Related Approach	Delivery Method	Main Outcomes
Ogawa et al. (2000) (15)	Preclinical	Mouse	Genetic infertility model	Spermatogonial stem cell (SSC) transplantation	SSC transplantation into seminiferous tubules	Restoration of spermatogenesis and fertility in infertile mice
Zohni et al. (2012) (22)	Preclinical	Mouse	Busulfan-induced testicular damage	Analysis of SSC recovery kinetics	—	Fertility restoration is dependent on SSC recovery after cytotoxic injury
Watanabe et al. (2018) (23)	Preclinical	Mouse testis	Blood–testis barrier-limited environment	In vivo genetic manipulation of SSCs and niche cells	AAV1/AAV9 microinjection	Efficient gene delivery beyond BTB: proof-of-feasibility for testicular gene transfer
Shinohara & Kanatsu-Shinohara (2020) (24)	Preclinical	Mouse SSCs → busulfan-treated recipients	Busulfan-prepared infertile testes	Lentiviral-mediated SSC transgenesis	Lentivirus pseudotyped with Sendai virus F protein + SSC transplantation	Stable genetic modification of SSCs and functional spermatogenesis
Azizi et al. (2021) (17)	Preclinical	Mouse	Busulfan-induced infertility	SSC transplantation	SSC transplantation	Resumption of spermatogenesis and improved fertility

				(cell-based restoration)		
Ogawa et al. (2003) (25)	Preclinical	Mouse	Local busulfan testicular injury	Recipient preparation for SSC-based interventions	Intratesticular busulfan injection	Effective depletion of germ cells for regenerative studies
Muramatsu et al. (1996) (20)	Preclinical	Mouse	Normal testis	In vivo gene transfer feasibility	Electroporation	Successful gene delivery to testicular cells
Michaelis et al. (2014) (21)	Preclinical (methods)	Mouse testis	Normal testis	Plasmid-based gene expression in the testis	Microinjection + electroporation	Reliable protocol for in vivo gene expression
J Anim Sci Biotechnol (2015) (26)	Preclinical	Mouse germline stem cells	Transgenesis context	Lentiviral-based gene delivery to MGSCs	Lentiviral vectors	Generation of transgenic offspring
Front Biosci (2025) (27)	Preclinical	Mouse testis	Gene-delivery optimization	Germ-cell targeted AAV variants	AAV2/9-based vectors	Superior germ cell transduction efficiency

Histological Recovery of Testicular Tissue

Histological outcomes were reported in seven studies, primarily those involving busulfan-induced testicular damage and subsequent SSC transplantation or genetic manipulation. Busulfan exposure resulted in marked degeneration of seminiferous tubules, loss of germinal epithelium, and depletion of endogenous germ cells (16, 17). Following SSC transplantation, several studies demonstrated repopulation of seminiferous tubules, restoration of germ cell layers, and re-establishment of spermatogenic architecture (15, 17, 19).

Watanabe et al. showed that AAV-mediated gene delivery could effectively transduce SSCs and somatic cells across the blood–testis barrier, supporting the feasibility of genetic manipulation within damaged testicular tissue(23). Collectively, these findings indicate that gene-based and cell-based interventions can support histological recovery in chemically compromised testes.

Restoration of Spermatogenesis and Sperm Production

Restoration of spermatogenesis was a primary outcome in six studies employing SSC transplantation into busulfan-treated recipients. Successful colonization of transplanted SSCs resulted in the resumption of complete spermatogenesis, including the production of mature spermatozoa (15-17, 19). Zohni et al. (2012) demonstrated that the efficiency of spermatogenic recovery was strongly dependent on the kinetics of SSC recovery following cytotoxic injury, highlighting the importance of stem cell niche restoration(22). Studies utilizing lentiviral-modified SSCs further showed that genetically manipulated germ cells retained their capacity for long-term spermatogenesis after transplantation (19). Although quantitative sperm parameters were inconsistently reported, multiple studies confirmed the presence of donor-derived sperm within the seminiferous tubules and epididymis.

Fertility Restoration

Functional fertility outcomes were evaluated in four studies using natural mating assays. Transplanting SSCs in busulfan-treated mice led to the successful restoration of fertility, as evidenced by the production of viable offspring (15, 17, 19). Ogawa et al. provided the first demonstration that SSC transplantation could restore fertility in infertile male mice,

establishing a foundational proof-of-concept for regenerative strategies targeting chemically damaged testes (25). Importantly, offspring derived from treated males were reported to be healthy and developmentally normal.

Gene Delivery Feasibility and Safety

Several studies specifically evaluated the feasibility and safety of gene delivery to testicular cells. AAV-based vectors demonstrated efficient transduction of SSCs and somatic cells without overt tissue damage or disruption of testicular architecture (18). Electroporation and microinjection techniques also enabled localized gene expression within testicular tissue, although with variable efficiency (20, 21). Across all included studies, no tumor formation, systemic toxicity, or gross testicular abnormalities related to gene delivery were reported. These findings suggest that gene-based manipulation of testicular cells is technically feasible and appears safe within preclinical settings.

Discussion

This systematic review provides a comprehensive synthesis of preclinical evidence on gene-based and gene-delivery–related strategies in models of chemically induced testicular injury. Although direct therapeutic gene therapy for chemical testicular damage has not yet reached clinical application, the available literature demonstrates converging evidence that genetic manipulation and gene delivery to testicular cells—particularly spermatogonial stem cells (SSCs)—are technically feasible and biologically meaningful.

Chemical agents such as busulfan and alkylating chemotherapeutics are well known to induce profound germ cell depletion and long-term infertility by targeting rapidly dividing spermatogonia (2, 4, 6). In line with these observations, the majority of included studies used busulfan-treated mouse models to establish a severe and reproducible form of testicular damage (16, 17). Consistent with early foundational work by Brinster and Zimmermann (28), transplantation of SSCs into chemically sterilized testes resulted in re-initiation of spermatogenesis and restoration of fertility (15, 17, 19).

Compared with earlier transplantation-only studies, more recent investigations have advanced the field by demonstrating the feasibility of genetic modification of SSCs before transplantation. Shinohara and Kanatsu-Shinohara showed that lentiviral-mediated transgenesis of SSCs preserved stem cell self-renewal and differentiation capacity following transplantation into busulfan-treated recipients (24). These findings align with broader SSC biology literature, emphasizing the robustness and plasticity of SSCs within a permissive niche (29, 30).

A major barrier to testicular gene therapy has historically been the blood–testis barrier (BTB), which restricts access of macromolecules to the adluminal compartment (31). However, Watanabe et al. demonstrated that AAV vectors can effectively transduce SSCs and somatic niche cells in vivo, challenging the notion that the BTB is an absolute obstacle to gene delivery (23). This observation is supported by methodological studies using electroporation and microinjection to achieve localized gene expression within testicular tissue (20, 21) and parallels advances in targeted drug and gene delivery across the BTB (8).

From a functional perspective, fertility restoration represents the most clinically relevant outcome. Several included studies reported successful generation of healthy offspring following SSC transplantation into chemically damaged testes (15, 17, 19), consistent with earlier demonstrations of donor-derived spermatogenesis and fertility restoration (28). Importantly, no overt developmental abnormalities were observed in offspring, although long-term multigenerational safety remains insufficiently studied. A mechanistic overview of the proposed gene-based regenerative pathways following chemically induced testicular injury is illustrated in Figure 2.

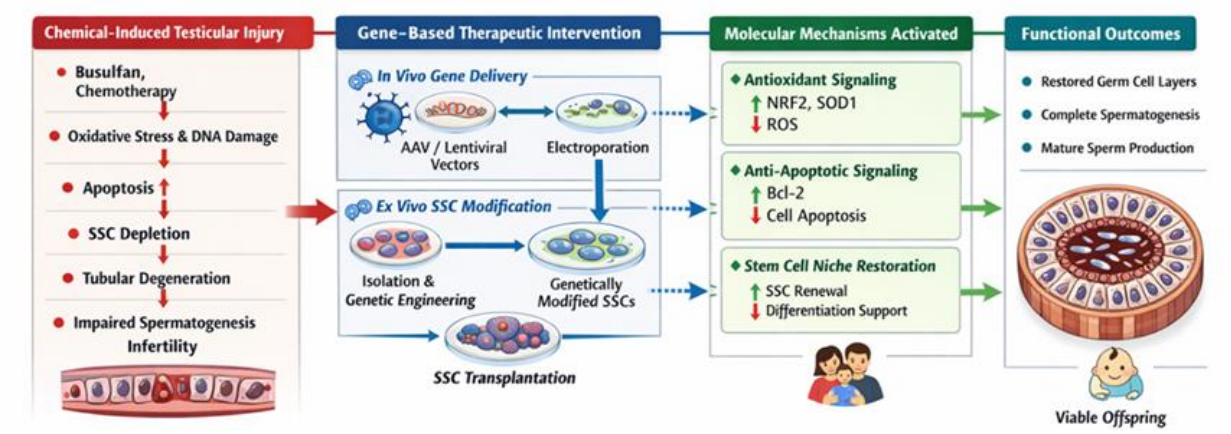

Figure 2. Mechanistic schematic illustrating gene-based therapeutic restoration of spermatogenesis following chemically induced testicular injury.

Despite these advances, significant limitations persist. All available evidence is preclinical, and most studies focus on feasibility rather than targeted correction of molecular pathways disrupted by chemical toxicity. Furthermore, broader concerns related to germline gene modification, insertional mutagenesis, and heritable genetic alterations—widely discussed in the gene therapy field—remain unresolved in the context of male infertility (7, 32).

In summary, when compared with earlier regenerative and transplantation studies, recent advances in gene delivery and SSC genetic manipulation represent a substantial conceptual progression. While direct gene therapy for chemically induced testicular injury has not yet been established, the existing literature provides a strong experimental foundation for future translational efforts that integrate targeted gene therapy with SSC-based regenerative platforms.

Conclusion

This systematic review highlights the promising role of gene therapy in restoring testicular structure and function following chemically induced injury. Preclinical evidence suggests that gene-based interventions can effectively improve testicular histology, enhance spermatogenesis, restore sperm parameters, and restore testosterone production, with some studies demonstrating the restoration of functional fertility. Therapeutic strategies targeting oxidative stress and apoptotic pathways appear particularly effective. However, clinical evidence remains limited, underscoring the need for well-designed human trials with standardized outcomes and long-term follow-up to establish the safety and translational applicability of gene therapy in the treatment of chemically induced male infertility.

Declarations

Ethics Approval and Consent to Participate

This study is a systematic review of previously published studies. No new human participants or animals were involved in this research. Therefore, ethical approval and informed consent were not required.

Consent for Publication

Not applicable.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article. The datasets analyzed are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

S.D. contributed to the conceptualization and design of the study, conducted literature screening, and drafted the manuscript. A.M. and M.T. performed the systematic search, data extraction, and quality assessment of the included studies. F.S. participated in the risk of bias evaluation and critically revised the manuscript. H.S. conceptualized and supervised the study, interpreted the data, critically revised the manuscript for important intellectual content, and acted as the corresponding author. All authors read and approved the final manuscript.

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Transparency Statement

The authors affirm that this manuscript is an honest, accurate, and transparent account of the study being reported, and that no important aspects have been omitted.

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