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Review

Plastic-Associated Pollutants and the Paternal Germline: From Reproductive Toxicity and Sperm Molecular Alterations to Potential Offspring Effects

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Abstract

Paternal preconception health is increasingly recognized as an important determinant of reproductive success and offspring development. Plastic-associated contaminants, including endocrine-disrupting chemicals, microplastics, and nanoplastics, have been increasingly associated with adverse effects on male reproductive function. The effects of these exposures may extend beyond conventional sperm quality, potentially inducing molecular changes in paternal germ cells with consequences for embryonic development and offspring health. This review examines how preconception exposure to plastic-associated pollutants may alter the male germline through oxidative stress, mitochondrial dysfunction, endocrine disruption, inflammation, and epigenetic alterations. Particular attention is given to DNA methylation, histone modifications, non-coding RNAs, and other epigenetic mechanisms that may contribute environmentally induced molecular information to the embryo. We will provide information about experimental and epidemiological research related to environmental exposure in males and the resulting impacts on fertility, development of embryos, placenta function, adverse pregnancy outcomes, and vulnerability of the offspring to reproduction, metabolic, neurodevelopmental, and cardiovascular diseases. By integrating evidence from reproductive biology, toxicology, developmental epigenetics, and environmental health, this review provides a framework for understanding how environmental exposures may alter the paternal germline and potentially influence health across generations. Finally, we identify key research gaps and methodological challenges, outline priorities for future research, and emphasize the need for comprehensive exposure assessment, mechanistic studies in humans, and strategies to reduce environmental exposures during the preconception period.

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1. Introduction

The paternal contribution to reproduction extends beyond the nuclear genome. Spermatozoa carry additional molecular information, including DNA methylation patterns, retained histones, and non-coding RNAs, that may regulate early embryonic development [1,2]. Experimental evidence indicates that paternal environmental exposures can alter these molecular features before fertilization. In mice, paternal dietary changes alter sperm small RNA profiles, while functional studies suggest that specific sperm RNAs regulate gene expression in the preimplantation embryo [3,4]. These observations allow us to consider the possibility of a biological mechanism by which paternal exposure to xenobiotics might affect reproductive and developmental processes through changes in the germline [5]. However, the fact that an exposure can change sperm molecular signatures does not mean that these changes play any biological role in reproductive or developmental processes or disease.

Plastic-associated pollutants represent an important class of environmental exposures in this context. These pollutants have distinct physicochemical properties and include bisphenols, phthalates, and micro- and nanoplastics [6]. Bisphenol A (BPA) is a widely used plastic-associated chemical that has been extensively investigated for its potential reproductive effects. Similarly, phthalates are known compounds, which are being studied because of their impact on the epigenetics of the sperm in humans [7,8]. Micro- and nanoplastics represent a distinct class of plastic-associated pollutants with specific toxicological properties that should not be generalized from those of plastic-associated chemicals [9]. These two categories should therefore be considered separately when evaluating their effects on reproductive health.

Human biomonitoring studies have further increased interest in the reproductive effects of plastic-related exposures. Microplastics have been found in human testicular tissue and semen, thereby proving the occurrence of plastic-related substances in male reproductive samples [10,11]. More recently, a multicenter study has described associations between microplastic diversity in male reproductive samples and decreased semen characteristics such as sperm concentration and progressive motility. However, these findings should be carefully interpreted [12,13]. The detection of microplastics does not provide any evidence on the exposure pathway, dose, or causal role in reproductive disorders. Additionally, the association with semen quality does not imply changes in epigenetic information of the sperm and its impact on the embryo [10].

Evidence linking plastic-associated chemicals to alterations in the sperm epigenome is more established, although their physiological relevance remains unclear. Exposure to BPA in occupational settings has been related to low levels of long interspersed nuclear element-1 (LINE-1) DNA methylation in sperm cells [14]. Another study of men undergoing in vitro fertilization (IVF) reported associations between urinary phthalate metabolite concentrations and sperm DNA methylation patterns [15]. These findings suggest that the human germline may be sensitive to chemical exposures; however, epidemiological studies cannot establish whether the observed molecular changes have functional consequences for reproductive outcomes. Experimental studies extend these observations to subsequent generations. In mice, paternal BPA exposure has been associated with altered sperm

function and DNA methylation, as well as reproductive abnormalities across multiple generations [16].

Therefore, distinguishing germline-mediated developmental programming from direct reproductive toxicity remains a major challenge. In this review, the term "paternal germline programming" refers to the potential process through which exposure-associated molecular alterations in paternal germ cells or sperm may influence subsequent developmental outcomes. The term does not imply that the detection of a sperm molecular alteration alone constitutes functional programming or demonstrates causal germline-mediated transmission. DNA damage, epigenetic alterations, and sperm dysfunction represent distinct biological endpoints that may coexist but differ in their potential implications for offspring. Similarly, an association between paternal exposure and an offspring phenotype does not necessarily indicate epigenetic inheritance. The distinction between intergenerational and transgenerational effects requires consideration of the timing and duration of exposure, the tissues and germ cells affected, and whether the observed effects persist beyond directly exposed generations [17,18]. Accordingly, adult paternal exposure before conception, developmental exposure of the future father, maternal or gestational exposure involving the developing male germline, and direct experimental exposure of sperm or testicular cells represent biologically distinct exposure scenarios and are considered separately in this review. These scenarios differ in the developmental stage and cell populations directly exposed and, consequently, in the interpretation of effects observed in subsequent generations. Compared with studies assessing isolated outcomes, experimental designs incorporating controlled paternal exposure, mating with unexposed females, molecular characterization of sperm alterations, and follow-up across subsequent generations provide a more robust framework for investigating these associations. Even within such experimental designs, functional evidence is required to establish whether a specific sperm alteration contributes causally to the observed offspring phenotype [19,20].

This narrative review critically appraises evidence suggesting that plastic-related pollutants may affect the paternal germline, reproductive outcomes, and offspring health. Previous reviews have addressed paternal environmental exposures and sperm epigenetics broadly, specific epigenetic mechanisms, endocrine-disrupting chemicals (EDCs), or the potential intergenerational effects of micro- and nanoplastics [21–25]. In contrast, the present review integrates evidence on plastic-associated chemicals, including bisphenols and phthalates, with the emerging literature on micro- and nanoplastics, while considering these exposure classes separately because of their distinct toxicological properties. Particular emphasis is placed on linking male reproductive effects and sperm molecular alterations with reproductive, embryonic, and offspring outcomes and on distinguishing associations and molecular continuity from evidence supporting germline-mediated intergenerational or transgenerational effects. This approach facilitates a critical appraisal of the strengths and limitations of the available evidence while helping to define priorities for future research.

2. Literature Search Strategy

This narrative review was developed to provide a critical synthesis of the relationship between paternal exposure to plastic-associated pollutants, sperm molecular and epigenetic alterations, and reproductive and offspring outcomes. Literature searches were conducted in PubMed/MEDLINE, Scopus, and Web of Science for publications available up to 1 October 2026. Search terms combined exposure-related concepts, including "BPA," "bisphenols," "phthalates," "plasticizers," "microplastics," and "nanoplastics," with paternal and reproductive terms such as "paternal exposure," "preconception," "male germline," "sperm," and "spermatogenesis." Additional terms addressed molecular mechanisms and developmental outcomes, including "epigenetics," "DNA methylation," "histone modifications," "chro-

matin," "non-coding RNA," "oxidative stress (OS)," "mitochondrial dysfunction," "embryo development," "offspring," "intergenerational," and "transgenerational." Terms were combined using Boolean operators and adapted to the search functions of each database. Reference lists of relevant primary studies and reviews were also examined to identify additional publications of direct relevance to the review.

Literature considered for inclusion comprised primary human observational studies, animal experiments, and in vitro investigations addressing plastic-associated exposures and at least one relevant endpoint: male reproductive function, sperm molecular or epigenetic characteristics, fertilization or embryonic development, or offspring outcomes. Reviews were used to provide background context and to identify relevant primary studies, whereas the interpretation of specific exposure–outcome relationships prioritized original research. Studies involving maternal or gestational exposure were considered separately when they provided relevant mechanistic or generational context and were not treated as equivalent to studies of paternal exposure before conception. Similarly, findings concerning plastic-associated chemicals were distinguished from those concerning plastic particles. Studies of other environmental exposures were considered only when necessary to provide broader mechanistic context for paternal germline regulation.

Exposure type, timing, route, dose or concentration, study population, and biological endpoints were considered in the synthesis. Studies were further classified according to exposure scenario, including adult paternal preconception exposure, developmental exposure of the future father, maternal or gestational exposure affecting the developing male germline, and direct experimental exposure of sperm or reproductive cells. Exposure window, experimental model, and generation examined were considered when interpreting intergenerational and transgenerational findings. The strength of evidence was further considered according to whether studies reported exposure-associated molecular alterations in sperm, embryo or offspring phenotypes, corresponding molecular alterations in sperm and descendants, functional evidence implicating a specific sperm-borne factor, or direct tests of causal mediation through targeted manipulation, transfer, inhibition, or rescue experiments. These categories were considered separately, as the coexistence of a sperm molecular alteration and an offspring phenotype does not, by itself, demonstrate that the alteration mediates the phenotype.

As a narrative review, this article was not designed to provide an exhaustive systematic inventory of the literature or a pooled estimate of effect. No formal systematic-review screening procedure or standardized risk-of-bias assessment was applied. Primary studies were selected based on their direct relevance to the review questions and their contribution to representing different exposure classes, exposure scenarios, experimental models, molecular mechanisms, and reproductive or offspring outcomes. Particular emphasis was placed on studies that provided human evidence, characterized sperm or germline alterations, assessed developmental or offspring outcomes, or included functional experiments relevant to potential germline-mediated effects. Differences in study design, exposure dose and duration, experimental model, exposure window, and outcome assessment were considered when comparing and interpreting findings across studies. Accordingly, the evidence discussed in the text and summarized in the tables should be regarded as a critical, selective synthesis of representative studies rather than a comprehensive or systematically evaluated catalogue of the available literature.

3. Plastic-Associated Pollutants and Paternal Exposure

Plastic-associated exposure includes not only chemicals involved in plastic manufacture or added to plastic-containing goods but also plastic particles [26,27]. Plastic-associated exposures vary in their physicochemical properties, biological fate, and measurement

approaches and thus should be studied individually before potential interactions are considered [28,29]. This review focuses primarily on bisphenols, phthalates, and micro- and nanoplastics, while considering other plastic-associated additives and chemicals relevant to mechanisms of paternal reproductive toxicity. The term "plastic-associated" does not imply that all chemicals originate exclusively from plastics or that the presence of plastic particles necessarily indicates exposure to specific additives [30].

3.1. Bisphenols and Phthalates

BPA is used in the production of polycarbonate plastics and epoxy resins and is among the most extensively studied plastic-associated chemicals in reproductive toxicology. Exposure can be found in occupational and general populations. In a study comparing occupationally exposed and unexposed men, higher urinary BPA levels were associated with poorer semen parameters, supporting further investigation of BPA as a potential male reproductive toxicant [31]. However, this kind of association alone does not pinpoint the biological mechanism behind the link.

BPA analogues used as substitutes, including bisphenol S (BPS) and bisphenol F (BPF), should also be considered. Population biomonitoring studies have documented exposure to BPS and BPF, suggesting that assessment of BPA alone may not adequately capture exposure to other bisphenols. Similar structure and wide detection are not sufficient to assume similar reproductive toxicity [32,33].

Phthalates represent yet another mixed group of exposures. Their applications range from plasticizers to components of various consumer products, with exposure routes and sources differing among individual chemicals. Data obtained from air and dust sampling in residential settings, as well as from surface wipes and hand wipes, exemplify how exposures occur in indoor environments [34–36]. Diet represents another relevant exposure route. In one dietary intervention aimed at reducing consumption of foods packaged in cans and plastics, urinary levels of BPA and bis(2-ethylhexyl) phthalate (DEHP) metabolites decreased [37,38].

Human reproductive studies generally assess phthalate exposure using urinary metabolites rather than the parent compounds [39]. A population-based study of men attempting to conceive reported significant associations between specific urinary phthalate metabolites and semen quality. It is important to note the uncertainty in the effect on fertility, however [40]. Interpretation should therefore consider the specific metabolites detected and their corresponding parent compounds. Population biomonitoring studies have documented exposure to BPS and BPF, suggesting that assessment of BPA alone may not adequately capture exposure to other bisphenols. Similar structure and wide detection are not sufficient to assume similar reproductive toxicity [41].

3.2. Microplastics and Nanoplastics

Micro- and nanoplastics represent particulate exposures rather than individual dissolved chemicals. Microplastics are generally defined as plastic particles smaller than 5 mm, whereas the size threshold distinguishing microplastics from nanoplastics remains inconsistent across studies [42–44]. Thus, the particle sizes reported in each study should be considered rather than relying solely on the terms "microplastics" and "nanoplastics".

Microplastics have been detected in testicular tissue and semen using different analytical methods. These studies demonstrate the presence of plastic-associated particles or polymers in male reproductive samples; however, their biological relevance depends on the biological compartment examined and the analytical methods employed [45,46]. Detection in testicular tissue or semen does not necessarily demonstrate access to the seminiferous compartment or confirm localization within spermatozoa [47]. Reported

concentrations also vary according to the analytical approach and sample characteristics. For example, microplastics have been reported at 0.23 ± 0.45 particles/mL in human semen and 11.60 ± 15.52 particles/g in testicular tissue, whereas a more recent semen study reported a mean abundance of 17.0 ± 42.0 particles/g, with particles ranging from 20.3 to 189.7 μm [13,45]. Such values should not be interpreted as an established biologically effective dose because particle number, mass, size, morphology, and polymer composition are not directly interchangeable across studies. Particle-resolved techniques such as Raman microspectroscopy, Fourier-transform infrared spectroscopy, and laser direct infrared spectroscopy can characterize polymer type and particle size, whereas pyrolysis–gas chromatography–mass spectrometry provides polymer-specific mass information but does not preserve particle-level characteristics [45,48]. Consequently, standardized sampling, contamination control, procedural blanks, and consistent reporting of particle abundance, size distribution, polymer composition, and analytical detection limits are essential for comparisons across human studies.

Human evidence relating quantified microplastic exposure to sperm dysfunction remains limited. Observational studies have reported polymer-specific differences or associations in progressive motility, although findings are not fully consistent [13,49]. In vitro exposure of human spermatozoa to polystyrene microplastics at 105 or 210 μg/mL decreased sperm motility and vitality and increased reactive oxygen species production and sperm DNA fragmentation. Altered expression of sperm genes involved in sperm–oocyte fusion, including DCST1, DCST2, IZUMO1, SPACA6, SOF1, and TMEM95, has also been reported [50]. More recent experimental evidence with polystyrene nanoplastics implicates mitochondrial OS and impaired mitochondrial function in human sperm dysfunction [51]. Nevertheless, these experimental concentrations cannot be directly equated with particle burdens measured in human semen, and direct human evidence linking quantified seminal microplastic exposure to a specific alteration in sperm metabolism remains insufficient. Moreover, no validated gene, protein, epigenetic, or metabolic signature can currently be considered specific to microplastic exposure in human sperm. OS, mitochondrial dysfunction, DNA damage, and alterations in sperm-function-related genes should therefore be considered candidate mechanistic responses rather than microplastic-specific biomarkers.

Experimental studies indicate that micro- and nanoplastic exposure may adversely affect male reproductive function under specific exposure conditions. In animal models, polystyrene microplastics have been associated with testicular inflammation and disruption of the blood–testis barrier, whereas oral exposure to polystyrene nanoplastics has been reported to induce testicular and sperm damage [52–54]. However, the relevance of these findings to human exposure depends on several factors, including particle characteristics, dose, exposure duration, and the experimental model employed [29,55]. Experimental evidence also indicates effects on the somatic testicular environment. Micro- and nanoplastic exposure has been associated with oxidative and mitochondrial stress in Leydig cells, impaired steroidogenic function, and reduced testosterone synthesis, while disruption of Sertoli-cell function and blood–testis barrier integrity may indirectly compromise germ-cell development [54,56]. Alterations in the LH/cAMP/PKA/StAR steroidogenic pathway and steroidogenic enzymes have been proposed in experimental models, although changes in circulating testosterone, LH, and FSH vary according to particle characteristics, dose, exposure duration, and model [54,57]. Human evidence establishing comparable endocrine effects remains insufficient. These hormonal and somatic-cell effects are important to distinguish from direct germline effects because impaired sperm quality following exposure may result from disruption of the testicular microenvironment rather than from a molecular alteration transmitted through sperm.

Species differences further complicate translation of these findings. Human spermatogenesis requires approximately 74 days, with a seminiferous epithelial cycle of approximately 16 days, whereas the corresponding cycle is approximately 8.6 days in mice and 12.8–12.9 days in rats; complete spermatogenesis is correspondingly shorter in rodents [58,59]. Spermatogonial organization also differs among species. Human spermatogonia have traditionally been classified into A_dark, A_pale, and type B populations, whereas rodents contain undifferentiated A_single, A_paired, and A_aligned populations followed by differentiating A1–A4, intermediate, and type B spermatogonia [60,61]. Therefore, an exposure period covering a substantial proportion or the entirety of spermatogenesis in a rodent does not represent an equivalent temporal exposure window in humans. Experimental microplastic studies further indicate effects across several testicular cell populations, including spermatogonia, spermatocytes, Sertoli cells, and Leydig cells [62–64]. This cellular heterogeneity makes it difficult to attribute an observed sperm phenotype to a single testicular target. These differences in spermatogenic organization, exposure timing, and cellular susceptibility should be considered when extrapolating experimental reproductive effects to humans.

A further question is whether micro- or nanoplastic-induced sperm alterations are responsible for developmental abnormalities in the offspring. Experimental paternal-exposure studies have reported sperm molecular or functional alterations together with impaired embryonic development or offspring phenotypes, and selected studies provide mechanistic support for sperm-associated pathways [51,65,66]. However, the coexistence of an altered sperm phenotype and an offspring phenotype do not by itself establish germline-mediated transmission. At present, no human study has demonstrated the complete causal sequence from quantified paternal micro- or nanoplastic exposure to a defined molecular or metabolic alteration in sperm and subsequently to an adverse embryonic or offspring phenotype. Prospective preconception studies combining standardized particle characterization with sperm multi-omics, functional reproductive endpoints, and longitudinal assessment of offspring will be required to evaluate this pathway.

Chemical and particulate exposures may be concurrent, but the roles of each should not be confused [30]. Particle studies need to take into account potential chemicals that may be released or co-exposure to these chemicals, and the measure of bisphenols or phthalate metabolites is not a measure of exposure to plastic particles [67,68]. Studies comparing each exposure could help clarify these issues.

3.3. Exposure Timing and Assessment

The timing of paternal exposure is an important consideration when evaluating potential reproductive effects. Exposures during adulthood may affect spermatogenesis, post-testicular sperm maturation, or sperm present near the time of conception, whereas developmental exposures may interfere with the establishment of reproductive function [69]. In zebrafish, BPA exposure at different stages of spermatogenesis induced distinct genetic and epigenetic alterations associated with different embryonic outcomes [70,71].

In human studies, exposure assessment should also consider the biological window relevant to the observed effect. Urinary BPA and phthalate levels may vary over time within individuals, and repeated-sampling studies have demonstrated temporal variability in phthalate metabolite excretion [72]. Hence, a single measurement taken close to semen collection might not fully represent the exposure during the time of sperm development and maturation [73,74].

Additional measurement challenges arise from specimen handling, contamination, analytical sensitivity, particle characteristics, and the exposure metric used. All of these aspects of the methodology are discussed in more depth in Section 8. Together, these

exposure-related factors provide the context for interpreting the reproductive toxicity and sperm molecular alterations discussed in the following sections.

4. Mechanisms of Paternal Reproductive Toxicity

Plastic-associated chemicals and micro- and nanoplastic particles may affect male reproduction through several partly overlapping mechanisms, including OS, mitochondrial dysfunction, altered steroidogenesis, disruption of the testicular microenvironment, and impaired sperm function. Although some of these mechanisms may overlap, chemical additives and plastic particles represent distinct exposure classes and should not be assumed to act through identical biological pathways. These mechanisms may affect somatic cells supporting spermatogenesis, developing germ cells, and mature spermatozoa. Their relative contribution depends on exposure conditions, dose, developmental stage, and the experimental model.

4.1. OS and Cellular Injury

Experimental evidence frequently implicates OS as a mechanism underlying the reproductive toxicity of plastic-associated exposures. Increased reactive oxygen species (ROS) production, impaired antioxidant defenses, or a combination of both may disrupt redox homeostasis and contribute to reproductive damage. In mice, polystyrene microplastic exposure has been shown to induce reproductive toxicity through OS and activation of the p38 MAPK signaling pathway [75]. Redox imbalance has also been implicated in phthalate-induced reproductive toxicity. For example, DEHP-induced testicular damage has been associated with OS and germ cell apoptosis, while its metabolite MEHP has produced similar redox alterations in experimental germ cell models. However, responses to these exposures may vary across reproductive cell types [76].

Studies involving direct exposure of human sperm provide further evidence of oxidative damage. In vitro exposure to BPA has been associated with OS, mitochondrial dysfunction, and apoptotic changes [77]. These findings suggest that BPA may directly affect mature sperm independently of endocrine-mediated mechanisms, although the magnitude and nature of these effects are likely to depend on the level of exposure.

OS can influence membranes, lipids, proteins, DNA integrity, and signaling pathways relevant to sperm functions [78,79]. Yet, OS often accompanies disorders of mitochondria, endocrinology, and inflammation; therefore, these systems cannot be considered independent biological pathways [75,80].

4.2. Mitochondrial Dysfunction

Mitochondrial damage may adversely affect reproductive function through alterations in ROS production, energy metabolism, and cellular injury pathways. These changes may be associated with reduced motility and viability in mature spermatozoa. Another experimental sperm model demonstrated reduced motility, viability, mitochondrial function, and intracellular ATP levels at higher BPA concentrations, whereas BPA exposure in human sperm was associated with pro-oxidant mitochondrial damage [77,81]. These findings suggest that mitochondrial damage may contribute to BPA-induced sperm toxicity.

OS and mitochondrial dysfunction may interact and reinforce one another. Complementary information on sperm damage may be obtained by assessing ATP levels, ROS production, viability, and mitochondrial membrane potential [82]. However, because spermatozoa can generate ATP through multiple metabolic pathways, reduced ATP levels do not necessarily indicate mitochondrial dysfunction [83,84].

In paternal exposure models, mitochondrial dysfunction should be interpreted in the broader context of sperm function and the reproductive cellular environment. Environ-

mentally induced mitochondrial damage in sperm should not be interpreted as evidence of direct transmission of paternal mitochondrial dysfunction, as paternal mitochondria are typically eliminated after fertilization in mammals [85,86].

4.3. Endocrine Signaling and Steroidogenesis

Altered steroidogenesis represents another mechanism through which exposure to plastic-associated pollutants may disrupt the hormonal milieu required for spermatogenesis. In vitro adult human testicular tissue cultures exposed to DEHP and MEHP had testosterone production disrupted, providing direct experimental evidence that phthalates can disrupt steroidogenesis in a human testicular model [87]. MEHP reduced cAMP production and impaired steroidogenesis in MA-10 Leydig cells by disrupting cholesterol transport, with OS also contributing to these effects [88]. These findings illustrate the mechanistic interplay between redox imbalance and endocrine disruption.

Disruption of steroidogenic signaling has been associated with particle exposure. Chronic exposure of mice to polystyrene microplastics decreased testosterone concentrations and altered the LH receptor-cAMP-protein kinase A-steroidogenic acute regulatory protein pathway [89]. These findings suggest that particles may interfere with steroidogenic and hormonal pathways, although their mechanisms of action may differ from those of conventional EDCs.

Dose and stage of development could also affect the steroidogenic response. Studies using rat Leydig cells at different stages of differentiation indicate that the effects of MEHP are influenced by both parameters [90,91]. Therefore, the developmental stage under investigation should be considered when interpreting findings from fetal, immature, and adult reproductive models [90–92].

4.4. Inflammation and Disruption of the Testicular Microenvironment

The microenvironment of the testis is yet another site where plastic-associated reproductive toxicity can take place. Exposure to polystyrene microplastics has caused testicular inflammation and the destruction of the blood-testis barrier in mice [54]. In rats exposed orally for 90 days, evidence included seminiferous tubule injury, impaired sperm parameters, apoptosis of spermatogenic cells, and reduced expression of barrier-related proteins. Other findings include OS, activation of the p38 MAPK pathway, and reduced nuclear factor erythroid 2-related factor 2 (Nrf2) [93,94].

Phthalates may also be detrimental to cellular interactions necessary for germ-cell development. Studies of DEHP and its metabolites showed that MEHP induced germ cell detachment in primary Sertoli–germ cell co-cultures [95]. Thus, reproductive toxicity may arise not only from direct damage to germ cells or spermatozoa but also from disruption of the cellular interactions required for normal spermatogenesis.

Blood–testis barrier disruption is particularly relevant because alterations in the seminiferous microenvironment can impair germ cell development [95]. Nonetheless, alterations in blood–testis barrier proteins or barrier integrity do not provide direct evidence that intact plastic particles reach germ cells.

4.5. Impaired Sperm Function and the Transition to Germline Programming

Effects of plastic-associated pollutants may extend to mature spermatozoa and their fertilizing capacity. In vitro exposure of human sperm to BPA altered sperm function and reduced protein tyrosine phosphorylation [96]. Chemicals with similar structures may nevertheless exert different biological effects. In an in vitro study of human sperm, BPS and BPF did not significantly impair mitochondrial function, motility, or viability under the tested conditions, in contrast to previously reported effects of BPA [77]. These results show

that the consequences of BPA cannot be directly applied to other bisphenols, but they do not suggest that these compounds are harmless.

The reproductive effects of plastic-associated pollutants involve OS, mitochondrial dysfunction, endocrine disruption, and inflammation, together with changes in the testicular microenvironment and sperm signaling [10,97]. However, the involvement of these processes does not necessarily indicate paternal germline programming. DNA damage and alterations in sperm chromatin, DNA methylation, or RNA profiles may coexist with cellular damage, but they do not necessarily reflect the same biological processes [98,99]. Evidence of germline programming requires demonstration that exposure-induced effects are associated with molecular alterations in sperm, whereas developmental programming additionally requires evidence that such alterations contribute to observed embryonic or offspring outcomes [100–102]. The potential epigenetic mechanisms underlying paternal germline programming are discussed in the following section.

The proposed pathways linking paternal exposure to plastic-associated pollutants with male reproductive toxicity, sperm molecular alterations, and potential developmental and offspring outcomes are summarized in Figure 1.

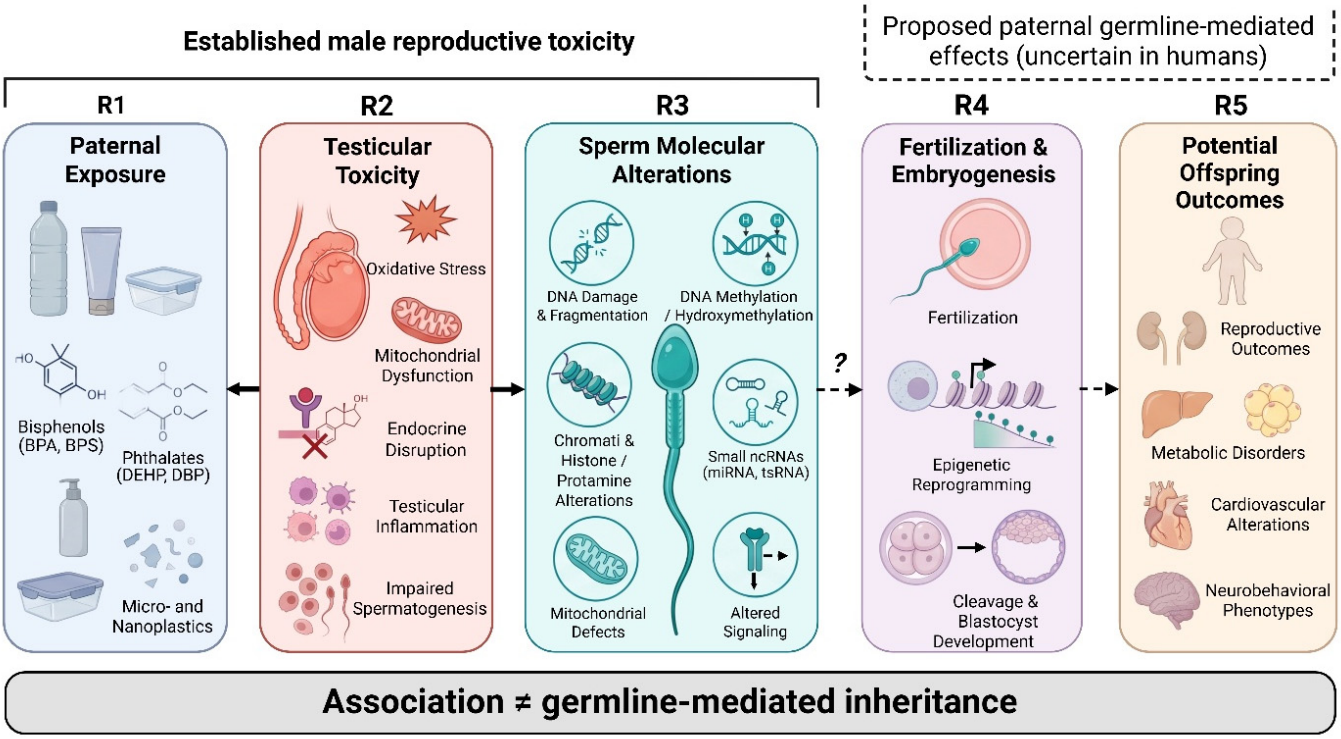


Figure 1. Potential pathways linking paternal exposure to plastic-associated pollutants with male reproductive and offspring outcomes. Created in BioRender. Potiris, A. (2026) https://BioRender.com/8kadhas.

5. Sperm Epigenetic Alterations and Potential Transmission

The sperm epigenome represents one possible route for interaction between paternal exposure and embryonic development. Some of the key elements in this regard are DNA methylation, histones and chromatin modifications associated with them, and non-coding RNAs. These elements vary in terms of their formation during spermatogenesis, environmental responsiveness, and post-fertilization fate [1]. Therefore, assessing their involvement in paternal programming would require not only detecting environmentally induced alterations but also establishing their links to early development or phenotypes [3,4].

5.1. DNA Methylation and Hydroxymethylation

DNA methylation is among the most extensively investigated epigenetic endpoints in studies of plastic-associated exposures and sperm. Human studies have associated BPA exposure with reduced LINE-1 methylation in sperm and genome-wide alterations in sperm DNA hydroxymethylation among occupationally exposed men [14,103,104]. These measures capture distinct aspects of the sperm epigenome: LINE-1 methylation reflects methylation at specific repetitive elements rather than across the entire sperm methylome, whereas DNA hydroxymethylation represents a distinct epigenetic modification.

Evidence from phthalate studies further supports the association between plastic-associated exposures and locus-specific alterations in sperm DNA methylation. Among men undergoing IVF, urinary concentrations of phthalate metabolites were associated with differentially methylated regions in sperm, some of which were also associated with blastocyst quality [15]. Although these findings do not establish whether the observed methylation differences mediate the relationship between paternal exposure and blastocyst quality, they provide a link between paternal exposure, alterations in the sperm methylome, and an early developmental outcome.

Controlled animal studies may provide further insight into the relationship between paternal exposure, sperm epigenetic alterations, and embryonic development. Adult male mice exposed to DEHP for 67 days and subsequently mated with unexposed females exhibited altered DNA methylation patterns in both sperm and embryonic tissues, together with changes in embryonic gene expression at embryonic day 7.5 [105]. However, only a limited number of differentially methylated regions were shared between sperm and F1 tissues, and the direction of methylation changes was preserved in only approximately half of the overlapping regions [105].

Interpretation of methylome data should also consider the functional relevance of the observed alterations. The presence of a differentially methylated region near a developmentally relevant gene does not necessarily indicate functional regulation of the corresponding developmental pathway [106]. Similarly, pathway enrichment analyses alone are insufficient to establish a causal developmental effect [107].

5.2. Histone Retention and Chromatin Organization

Although most of the sperm genome is packaged by protamines, a small proportion remains associated with histones. In human sperm, retained nucleosomes and histone modifications have been identified at specific developmentally relevant loci, including imprinted regions and promoters of developmental genes [108]. These findings suggest that sperm chromatin retains regulatory features beyond the DNA sequence that may be susceptible to environmental exposures.

Plastic-associated exposures may also alter sperm chromatin organization. In zebrafish, BPA exposure at different stages of spermatogenesis was associated with changes in histone acetylation and chromatin integrity, together with alterations in embryonic development and survival [71]. However, because histone alterations occurred alongside DNA damage, the observed developmental effects cannot be attributed specifically to chromatin modifications.

Further zebrafish studies have examined histone acetylation in embryos derived from BPA-exposed males. Increased acetylation of histone H3 at lysine 9 (H3K9ac) at specific regulatory regions was accompanied by altered expression of genes involved in cardiogenesis. Treatment of the embryos with epigallocatechin gallate (EGCG), which can inhibit histone acetyltransferase activity, restored both acetylation levels and gene expression [109]. Although these findings do not demonstrate direct inheritance of sperm histone modifications, they support a potential role for acetylation-dependent regulation in the observed developmental effects.

Histone-related analyses should distinguish among total histone retention, nucleosome organization, and post-translational histone modifications, as these represent distinct but interrelated features of sperm chromatin [108]. Similarly, findings obtained directly from mature sperm should be distinguished from those derived from whole testicular tissue [70,71].

5.3. Non-Coding RNAs and Epididymal Remodeling

Another possible means by which environmental information from fathers can affect developmental processes is via the sperm non-coding RNAs. This class of RNA undergoes reprogramming during spermatogenesis and epididymal transit. In mice, tissue-specific metabolic labeling demonstrated that mature sperm contain RNAs originating from the epididymal epithelium, supporting post-testicular RNA transfer from somatic cells to sperm [110].

Functional studies have demonstrated the developmental relevance of specific sperm RNAs. In mice, analysis of sperm from different regions of the epididymis identified embryonic gene expression changes mediated by sperm-delivered small RNAs, and these molecular and developmental abnormalities were rescued by supplementation with cauda-enriched small RNAs [111]. Further research, however, has shown that both caput and cauda sperm can sustain complete development in other strains of mice under different experimental conditions [112,113].

Evidence of pollutant-specific effects comes from a mouse study in which paternal DCHP exposure was associated with altered sperm small RNA profiles and metabolic abnormalities in the offspring. Using PANDORA-seq, the authors identified alterations in transfer RNA- and ribosomal RNA-derived small RNAs that were not detected by conventional RNA sequencing methods [114]. This example demonstrates the impact of RNA modifications and RNA library preparation on the detection of the sperm RNA signature. The causative role of the identified RNA changes needs to be confirmed [114].

Crucially, RNA-mediated effects do not necessarily require the persistent presence of the original paternal RNA. Paternally transmitted RNAs may act transiently during early development, initiating downstream regulatory events with potentially longer-lasting effects [115,116]. Physiologically appropriate RNA amounts are especially important when testing potential RNA-mediated processes.

5.4. Epigenetic Reprogramming and Evidence for Transmission

The extensive epigenetic reprogramming that occurs after fertilization represents a major challenge when interpreting exposure-induced alterations in the sperm epigenome [5]. In mice, post-fertilization reprogramming involves substantial remodeling of paternal DNA methylation through TET3-mediated oxidation of 5-methylcytosine followed by replication-dependent demethylation [117,118]. However, this process is not uniform across the genome, as certain regions, including imprinted loci, are protected from demethylation during epigenetic reprogramming [119,120]. Consequently, an exposure-associated DNA methylation pattern detected in sperm should not be expected to be transmitted unchanged to the offspring.

Thus, evidence for germline-mediated developmental effects can be evaluated at several levels. Exposure-associated molecular alterations in sperm indicate a germline response but do not establish developmental consequences, whereas embryo or offspring phenotypes following paternal exposure demonstrate a developmental association without identifying the mediating mechanism. Detection of corresponding molecular alterations in sperm and descendants strengthens evidence for biological continuity but does not establish mediation. Stronger evidence requires functional testing of a candidate sperm-borne factor, while direct manipulation, transfer, inhibition, or rescue experiments can provide more specific evidence that the candidate alteration contributes to the developmental phenotype [4,111]. As discussed in Section 7, the interpretation of intergenerational effects requires additional considerations. The exposure scenario is also critical to this interpretation. Adult paternal preconception exposure directly involves the paternal organism and germline, whereas developmental or gestational exposure may involve different stages of germ-cell development and additional directly exposed generations. Direct in vitro exposure of sperm or reproductive cells can provide mechanistic information but should not be considered equivalent to an in vivo paternal exposure model.

In summary, several sperm-borne molecular mechanisms may contribute to the developmental effects of paternal exposures, including alterations in DNA methylation, histone-associated chromatin regulation, and non-coding RNA profiles [1]. For plastic-associated exposures, the evidence is stronger for exposure-induced molecular alterations in sperm and their potential mechanistic relevance in experimental models than for a causal contribution of sperm-borne signals to health outcomes in human offspring. Moreover, the evidence supporting reproductive toxicity from micro- and nanoplastic particles is currently weighted toward cellular and tissue-level mechanisms, whereas bisphenol and phthalate studies provide a more developed literature on exposure-associated alterations in the sperm epigenome.

Table 1 summarizes representative primary studies investigating molecular and epigenetic alterations in the paternal germline following exposure to plastic-associated chemicals and particles. Collectively, these studies report changes in sperm DNA methylation and hydroxymethylation, histone modifications, chromatin organization, and short non-coding RNAs. However, the strength of functional evidence linking these molecular alterations to the transmission of offspring phenotypes varies considerably across studies.

Table 1. Representative primary studies linking plastic-associated environmental exposures to molecular and epigenetic alterations in the paternal germline. Abbreviations: BPA, bisphenol A; BPS, bisphenol S; DEHP, di(2-ethylhexyl) phthalate; DCHP, dicyclohexyl phthalate; DBP, dibutyl phthalate; DINCH, diisononyl cyclohexane-1,2-dicarboxylate; DMR, differentially methylated region; DhMR, differentially hydroxymethylated region; 5hmC, 5-hydroxymethylcytosine; CTCF, CCCTC-binding factor; PS-MP, polystyrene microplastic; PS-NP, polystyrene nanoplastic; PL, protamine-like protein; tsRNA, tRNA-derived small RNA; rsRNA, rRNA-derived small RNA; IVF-ET, in vitro fertilization and embryo transfer; EGCG, epigallocatechin gallate; DSB, DNA double-strand break; F0–F3, filial generations.

Exposure	Study/Model	Exposure Scenario and Window	Sperm/Germline Endpoint	Molecular/Epigenetic Alteration	Mechanistic/Functional Evidence	Generation(s)	Key Limitation	Reference
BPA	Occupationally exposed men	Human occupational exposure: 149 workers (77 BPA-exposed, 72 controls); urinary BPA assessment	Mature sperm DNA methylation	LINE-1 hypomethylation in sperm; association not observed in peripheral blood	No functional experiment; adjustment for sperm LINE-1 methylation did not explain the BPA–semen-quality association	F0 sperm only	Occupational observational design; LINE-1 is a global surrogate rather than locus-specific epigenetic profiling; no offspring assessment	[14]
BPA	Occupationally exposed men	Human occupational exposure: 30 BPA-exposed and 26 unexposed men	Mature sperm DNA hydroxymethylation	Genome-wide increase in 5hmC; 9610 DhMRs (8670 hyper-, 940 hypo-hydroxymethylated), involving 2008 genes and including LINEs and imprinted/sperm-expressed genes	None; epigenomic association only	F0 sperm only	Small occupational cohort; pooled sperm DNA for sequencing prevented individual-level epigenome–exposure analysis; relatively high exposure	[104]

Table 1. *Cont.*

Exposure	Study/Model	Exposure Scenario and Window	Sperm/Germline Endpoint	Molecular/Epigenetic Alteration	Mechanistic/Functional Evidence	Generation(s)	Key Limitation	Reference
BPA	Zebrafish	Adult paternal preconception exposure: F0 males exposed to 100 or 2000 µg/L during early spermatogenesis; mating with unexposed females	Sperm histone acetylation	Sperm histone hyperacetylation transmitted to F1 blastomeres; F1 enrichment of H3K9ac at kat6a, esr2b and hand2	EGCG treatment normalized histone acetylation and gene expression and rescued the cardiac phenotype, providing functional rescue evidence supporting a role for altered acetylation	F1 intergenerational	Zebrafish model; F1 only; pharmacological intervention is not locus-specific	[109]
BPA	Mouse	Gestational ancestral exposure: BPA exposure followed through multiple unexposed generations	Sperm chromatin accessibility/regulatory architecture	Persistent altered chromatin accessibility and CTCF-associated regulation at an Fto enhancer in descendant sperm	Functional deletion/disruption of the implicated CTCF-binding region prevented the ancestral BPA-associated obesity phenotype, providing direct functional evidence supporting the implicated locus-specific mechanism	F1–F6; F3 first transgenerationally eligible	Gestational exposure initially exposes both F1 fetus and developing F2 germline; locus-specific mechanism may not represent genome-wide transmission	[121]

Table 1. *Cont.*

Exposure	Study/Model	Exposure Scenario and Window	Sperm/Germline Endpoint	Molecular/Epigenetic Alteration	Mechanistic/Functional Evidence	Generation(s)	Key Limitation	Reference
BPA/BPS	Mouse	Gestational exposure followed across descendant generations	Neonatal male germ-cell transcription/chromatin accessibility; later sperm phenotype	Single-nucleus RNA/ATAC profiling identified persistent exposure-associated alterations in spermatogonial transcriptional and chromatin-accessibility programs	Multi-omic concordance across generations; no direct transfer/rescue establishing a specific molecular mediator	F1–F3; F3 first transgenerationally eligible	Germline molecular profiling centered on neonatal spermatogonia rather than mature sperm; causal molecular mediator not established	[122]
BPA	Rare minnow (*Gobiocypris rarus*)	Adult paternal preconception exposure: F0 males exposed to 15 µg/L for 14 d (measured 14.03 ± 0.89 µg/L), then mated with untreated females	Mature sperm miRNAs	28 sperm miRNAs altered (13 up, 15 down); aca-miR-16a-5p proposed in relation to *bmp2a* and craniofacial development	No RNA-transfer, inhibition, or rescue experiment	F1 intergenerational; F2 first transgenerationally eligible but phenotype absent	Candidate miRNA mechanism remains associative; fish model; lack of persistence into F2	[123]

Table 1. *Cont.*

Exposure	Study/Model	Exposure Scenario and Window	Sperm/Germline Endpoint	Molecular/Epigenetic Alteration	Mechanistic/Functional Evidence	Generation(s)	Key Limitation	Reference
Phthalates and alternatives	Human LIFE, SEEDS and EARTH cohorts	Human preconception exposure: 697 men; 18 phthalate and 2 alternative metabolites measured in preconception urine	Mature sperm DNA methylation (EPIC 850K)	7979 exposure-associated DMRs identified in at least one cohort; meta-analysis identified 946 MBzP-, 27 MiBP-, and 1 MEHP-associated DMRs; enrichment in spermatogenesis, hormonal and developmental pathways	None; observational epigenome-wide association	F0 sperm only	Single urine measurements may incompletely represent the spermatogenic exposure window; cohort/sample-processing heterogeneity; no causal transmission assessment	[7]
DEHP	Mouse C57BL/6J	Adult paternal preconception exposure: F0 males, 2.5 or 25 mg/kg for 67 d (~2 spermatogenic cycles); mating with unexposed females	Sperm DNA methylation	704 sperm DMRs; F1 E7.5 embryos and extraembryonic tissues showed 1716 and 3181 DMRs, respectively; 29 DMRs overlapped sperm and F1 tissues, with approximately half concordant	Molecular continuity between paternal sperm and F1 tissues, accompanied by altered F1 transcription; no direct methylation transfer/rescue experiment	F1 intergenerational	Overlapping DMRs do not demonstrate causal mediation; early developmental endpoints rather than mature offspring phenotype	[105]

Table 1. *Cont.*

Exposure	Study/Model	Exposure Scenario and Window	Sperm/Germline Endpoint	Molecular/Epigenetic Alteration	Mechanistic/Functional Evidence	Generation(s)	Key Limitation	Reference
DEHP	Mouse	Developmental exposure of future fathers: prenatal exposure to 300 mg/kg/day DEHP	Adult sperm transcriptome and DNA methylation	Persistent changes in sperm RNA and promoter methylation, including seminal-vesicle secretory protein-related loci and miR-615-associated regulation	Molecular associations between developmental exposure and adult germline state; no direct transfer experiment	Developmentally exposed F1 germline	High experimental dose; exposure occurred during development of future fathers rather than adult preconception period; causal transmission not established	[124]
DEHP	Mice C57BL/6J and FVB/N	Developmental exposure of future fathers: prenatal 300 mg/kg/day DEHP; susceptible and resistant genetic backgrounds compared	Sperm RNA/methylation and sperm functional phenotype	Strain-dependent sperm molecular alterations including Svs3ab-associated promoter methylation/seminal-vesicle protein pathway changes	Persistence of sperm phenotype across generations differed according to genetic background, demonstrating host-genotype modification	F1–F3; F3 first transgenerationally eligible	High prenatal dose; molecular changes not functionally demonstrated to mediate transmission	[125]

Table 1. *Cont.*

Exposure	Study/Model	Exposure Scenario and Window	Sperm/Germline Endpoint	Molecular/Epigenetic Alteration	Mechanistic/Functional Evidence	Generation(s)	Key Limitation	Reference
DBP/phthalates	Mouse	Adult paternal preconception exposure: phthalate exposure during spermatogenesis/preconception	RNAs carried and delivered by sperm at fertilization	Exposure altered sperm-delivered RNAs and an interconnected CRREW epigenetic-regulatory network relevant to early embryonic regulation	Direct assessment of sperm RNA cargo delivered to the fertilized oocyte strengthens biological plausibility; specific RNA-mediated offspring phenotype not isolated by transfer/rescue	F0 sperm/F1 early embryo	Complex RNA network; individual causal sperm-RNA species and long-term offspring consequences not established	[126]
DCHP	Mouse	Adult paternal preconception DCHP exposure followed by mating with unexposed females	Sperm small non-coding RNA profile	Broad remodeling of sperm small RNAs, associated with metabolic abnormalities in descendants	Sperm small-RNA analyses support a candidate transmission pathway, but individual RNA contribution to descendant phenotype was not definitively established	F1 intergenerational; F2 first transgenerationally eligible	Specific causal RNA species/mechanism remains unresolved	[114]

Table 1. *Cont.*

Exposure	Study/Model	Exposure Scenario and Window	Sperm/Germline Endpoint	Molecular/Epigenetic Alteration	Mechanistic/Functional Evidence	Generation(s)	Key Limitation	Reference
DEHP	Rat	Adult paternal preconception exposure: F0 males exposed orally to 0.1, 0.5 or 1.0 mg/kg/day for 15 wk before mating with unexposed females	Sperm miRNAs	Ten consistently downregulated sperm miRNAs; miR-10a-5p identified as key candidate; downstream Klf11–Kit/Ins2 regulatory axis	Functional manipulation of miR-10a-5p by inhibitor microinjection into normally fertilized zygotes phenocopied the male metabolic abnormalities; molecular targeting and knockdown experiments supported the Klf11 pathway	F1 intergenerational; F2 first transgenerationally eligible	Functional experiment phenocopied reduced miR-10a-5p but did not constitute complete rescue of the DEHP-exposed sperm phenotype	[127]
Phthalates/DINCH	Human SEEDS cohort	Human preconception exposure: 48 men undergoing IVF; preconception urinary metabolites	Mature sperm DNA methylation (450K array)	131 unique sperm DMRs; MEHP associated with the largest number (83); 13% of DMRs inversely associated with high-quality blastocyst development	Association of sperm DMRs with embryo quality provides developmental relevance, but no mediation experiment	F0 sperm/F1 embryo outcome	Small cohort; single spot urine measurement; observational ART population; DMR–blastocyst association does not establish mediation	[15]

Table 1. *Cont.*

Exposure	Study/Model	Exposure Scenario and Window	Sperm/Germline Endpoint	Molecular/Epigenetic Alteration	Mechanistic/Functional Evidence	Generation(s)	Key Limitation	Reference
PS-NPs	Zebrafish	Direct adult reproductive exposure: adults exposed to 45–50 nm PS-NPs, 5 mg/L for 96 h	Germ-cell internalization, sperm chromatin and testicular transcriptome	PS-NPs crossed the testicular barrier and entered germ cells; abnormal sperm chromatin compaction; altered meiosis/DSB/chromosome-organization pathways; 2303 testicular DEGs	Direct evidence of particle entry into germ cells; no functional experiment linking particle internalization or sperm alterations to an offspring phenotype	F0 germline	RNA-seq performed in whole testis, not purified sperm; sperm chromatin phenotype not epigenomically characterized; both sexes exposed in offspring assay, preventing paternal attribution	[128]
PS-MPs	Mouse	Adult paternal preconception exposure: F0 males exposed to 1.1-μm PS, 10 mg/kg/day by gavage for 4 wk, then mated with unexposed females	Sperm small RNAs assessed by PANDORA-seq	Altered tsRNA and rsRNA profiles in sperm	Selected exposure-responsive sperm RNAs altered gene expression in mouse embryonic stem cells, supporting regulatory activity but not in vivo mediation	F1 intergenerational	No sperm-RNA transfer/rescue demonstrating causation in offspring; metabolic phenotype was sex-specific and particularly apparent under dietary challenge	[65]

Table 1. *Cont.*

Exposure	Study/Model	Exposure Scenario and Window	Sperm/Germline Endpoint	Molecular/Epigenetic Alteration	Mechanistic/Functional Evidence	Generation(s)	Key Limitation	Reference
PS-NPs	Mouse	Adult paternal preconception exposure: F0 males exposed to 80 nm PS-NPs, 10 mg/kg/day orally for 60 d; mating with unexposed females; paternal lineage followed to F3	Sperm DNA methylation	Predominant sperm hypermethylation, including FtMt promoter hypermethylation; FtMt CpG4/5 hypermethylation also present in F1 sperm but absent in F2 sperm	IVF-ET with exposed F0 sperm reproduced F1 pulmonary injury and FtMt methylation; zygotic Dnmt3a knockdown reduced FtMt promoter methylation, providing functional evidence supporting the proposed sperm-originating mechanism	F1 intergenerational; F2 first transgenerationally eligible; F3 phenotype absent	Dnmt3a manipulation was not locus-specific sperm methylation editing; therefore definitive inheritance of the FtMt mark itself is not established	[66]
PS-MPs	*Mytilus galloprovincialis*	Direct adult reproductive exposure: adult mussels exposed for 48 h to 5-µm PS-MPs at 0.5 or 1 µg/L	Sperm protamine-like proteins/chromatin	Altered PL electrophoretic properties, increased PL–DNA binding/protection and reduced PL release at 1 µg/L; pronounced poly(ADP)-ribosylation of PL-II and PARylation of histone H1	Biochemical EMSA, DNA-protection and sperm-nuclear-protein release assays demonstrate altered PL–DNA interactions	F0 sperm only	Acute marine-invertebrate model; no fertilization/offspring experiment; MNase accessibility not significantly altered; no evidence of transmission	[129]

6. Evidence for Embryonic, Pregnancy, and Offspring Outcomes

The developmental relevance of paternal exposure depends on whether reproductive or sperm molecular alterations are associated with embryonic, pregnancy, or offspring outcomes [130,131]. All three endpoints represent distinct stages in the reproductive process and need to be assessed independently. Human studies have data from field conditions, while experimental models allow more control over exposure and developmental assessment [132,133].

6.1. Fertilization and Embryonic Development

Studies of couples undergoing assisted reproduction provide an opportunity to examine paternal exposures in relation to clinically relevant reproductive outcomes, including fertilization, embryo quality, implantation, and live birth. In the Environment and Reproductive Health (EARTH) study, paternal urinary phthalate metabolite concentrations were evaluated in relation to reproductive outcomes, with certain metabolites associated with less favorable outcomes [134–136]. However, associations varied across individual metabolites and reproductive endpoints, limiting the extent to which these findings can be generalized across phthalates as a chemical class.

Sperm DNA methylation may provide a biologically plausible link between paternal phthalate exposure and early developmental outcomes. As discussed in Section 5, urinary phthalate metabolite concentrations were associated with differentially methylated regions (DMRs) in sperm, some of which were also associated with blastocyst quality. However, embryo morphology represents an intermediate reproductive endpoint and does not establish subsequent developmental or offspring health effects.

Experimental evidence further indicates that exposure-induced molecular alterations are not necessarily accompanied by measurable developmental phenotypes. In mice, paternal DEHP exposure induced molecular changes in sperm and embryos without affecting overall developmental outcomes during gastrulation [105]. By contrast, paternal BPA exposure in zebrafish was associated with sperm alterations as well as impaired embryonic development and reduced embryo survival [71]. Together, these findings indicate that molecular alterations in the paternal germline do not invariably translate into detectable developmental effects.

6.2. Pregnancy Outcomes and Extraembryonic Development

A possible link exists between paternal exposures and events that occur after implantation. A factor analysis within the EARTH study associated a paternal exposure profile enriched in DEHP metabolites with an increased risk of treatment failure before live birth, with stronger associations observed after implantation [135]. As the PCA-derived factor represents a pattern of correlated biomarkers, the contribution of individual metabolites cannot be distinguished from this analysis.

There is more conflicting evidence about gestational length. The EARTH study found that the relationship between paternal concentrations of DEHP metabolites and preterm birth was weakened to the null after controlling for maternal preconception exposure to phthalates and phthalate alternatives [137]. Another mixture analysis suggested that paternal MEHP exposure may contribute to associations observed for combined parental exposure patterns. These findings illustrate the difficulty of distinguishing paternal exposure effects from correlated maternal exposures [138].

Experiments have similarly shown that extraembryonic tissues can react to paternal exposure. In mice, paternal DEHP exposure was associated with altered DNA methylation and gene expression in extraembryonic tissues during early development. These molecular alterations indicate a biological response to exposure but do not necessarily imply placental dysfunction [105].

6.3. Reproductive Health of Offspring

Evidence from experimental models suggests that paternal exposure may affect reproductive function in descendants. Adult paternal BPA exposure in mice resulted in abnormal spermatogenesis, sperm function, and fertility in several generations of descendants, as well as changes in sperm DNA methylation [16]. The persistence and magnitude of individual effects varied according to the endpoint, exposure dose, and generation.

Reproductive phenotypes should also be interpreted according to whether they represent tissue or molecular alterations, reproductive function, or fertility outcomes. Testicular histology, reproductive hormone concentrations, and semen parameters can indicate alterations in reproductive physiology [139]. In contrast, mating performance, successful conception, and offspring production provide more direct measures of reproductive capacity. The generational interpretation of these effects is discussed in Section 7.

6.4. Metabolic Outcomes

Paternal exposure has also been associated with metabolic phenotypes in offspring. As discussed in Section 5, paternal DCHP exposure in mice was associated with alterations in sperm small RNA profiles and metabolic abnormalities in the offspring [114]. Although the mechanistic relationship between these findings remains unclear, their co-occurrence provides a basis for investigating the potential role of sperm-borne RNAs in mediating developmental effects.

Evidence from particle-specific studies further extends these observations to polystyrene microplastics. Park et al. exposed male mice to 1.1 µm polystyrene microplastics by oral gavage at 10 mg/kg body weight/day for four weeks before mating them with unexposed females [65]. Paternal exposure was associated with sex-dependent alterations in offspring body composition following a high-fat diet challenge, while impaired insulin tolerance was observed in female offspring; glucose tolerance was not significantly affected. The exposure also altered sperm small RNA profiles, including transfer RNA- and ribosomal RNA-derived small RNAs [65,114]. In mouse embryonic stem cells, selected RNA species influenced the expression of specific genes, providing functional evidence that exposure-associated sperm RNA alterations may have regulatory effects [65]. Therefore, although this study does not establish an in vivo mediating role for the identified RNAs, it links paternal particle exposure with offspring metabolic phenotypes and alterations in candidate sperm RNAs. Crucially, these metabolic effects were assessed in response to a nutritional challenge rather than under baseline metabolic conditions.

The developmental period of the future male parent represents a distinct window of susceptibility to environmental exposures. In a rat model, future fathers were exposed to BPA during gestation and lactation through maternal exposure. Alterations in sperm Igf2 methylation were subsequently detected in these males, while pancreatic dysfunction and Igf2-related epigenetic alterations were observed in their offspring [140]. This experimental design therefore addresses developmental programming of the future paternal germline rather than exposure occurring during adulthood.

6.5. Neurobehavioral and Cardiac Developmental Outcomes

Paternal behavioral impacts of BPA exposure have been assessed experimentally. In rodents, preconception paternal BPA exposure has been associated with changes in behavioral reactivity, spatial memory, and related outcomes. The direction and magnitude of these effects may depend on factors such as sex, age, experimental paradigm, and exposure conditions, while behavioral changes in animal models should not be equated with clinical neurodevelopmental impairment [141,142].

Human cohort studies have also examined associations between parental preconception exposure to phthalates and BPA and behavioral outcomes in children, with exposure assessed in both parents [143]. Although questionnaire-based behavioral measures are distinct from clinical diagnoses and may be influenced by shared parental and environmental factors, these findings extend the available evidence to human offspring.

Experimental studies have also examined cardiac development in zebrafish. As discussed in Section 5, paternal BPA exposure was associated with impaired cardiac development in embryos and alterations in histone acetylation at regulatory regions of genes involved in cardiogenesis [109]. It is important to distinguish such mechanistic evidence from evidence of clinically manifest cardiovascular disease in human offspring.

Table 2 summarizes representative primary studies examining associations between paternal exposure to plastic-associated chemicals and particles and embryonic, pregnancy, and offspring outcomes. The available studies include reproductive, metabolic, neurodevelopmental, cardiopulmonary, and early developmental phenotypes and, where studied, potential mediators carried by the sperm.

Table 2. Developmental, offspring, and multigenerational outcomes following paternal exposure to plastic-associated environmental contaminants. Abbreviations: BPA, bisphenol A; BPF, bisphenol F; BPS, bisphenol S; DEHP, di(2-ethylhexyl) phthalate; DCHP, dicyclohexyl phthalate; DMR, differentially methylated region; EGCG, epigallocatechin gallate; FtMt, mitochondrial ferritin; IVF, in vitro fertilization; miRNA, microRNA; ncRNA, non-coding RNA; NMN, nicotinamide mononucleotide; PS-NP, polystyrene nanoplastic; ROS, reactive oxygen species; rsRNA, ribosomal RNA-derived small RNA; tsRNA, tRNA-derived small RNA.

Exposure	Study/Model	Exposure Scenario and Window	Generation	Developmental/Offspring Outcome	Candidate Sperm Mediator	Main Findings	Key Limitation	Reference
BPA	Rat	Developmental exposure of future fathers: F0 dams received BPA (40 µg/kg/day) from GD0–PND21; developmentally exposed F1 males were subsequently mated with untreated females	F2	Impaired glucose tolerance and pancreatic β-cell dysfunction, particularly in males	Igf2 DMR2 DNA methylation	F1 paternal sperm showed increased Igf2 DMR2 methylation and reduced Igf2 expression; related hypermethylation was detected in F2 male pancreatic islets together with metabolic dysfunction	F1 fathers and their developing germ cells were directly exposed; therefore, F2 represents an intergenerational/multigenerational, not definitive transgenerational, effect. Molecular continuity does not establish causal mediation	[140]
BPA	Mouse	Adult paternal preconception exposure: F0 males received BPA (5 or 50 mg/kg/day) for 6 weeks before mating with untreated females; paternal-line breeding continued without further BPA exposure	F1–F3	Reduced sperm quality and impaired IVF cleavage/blastocyst development across descendants; some effects persisted into F3	Altered global sperm DNA methylation (5-mC)	Sperm count, motility/hyperactivation, ATP and embryo-developmental competence were impaired across generations to varying degrees; sperm 5-mC remained elevated through F3	High experimental doses; global 5-mC is nonspecific; no transfer or epigenetic editing demonstrating causal mediation; several phenotypes attenuated across generations	[16]

Table 2. *Cont.*

Exposure	Study/Model	Exposure Scenario and Window	Generation	Developmental/Offspring Outcome	Candidate Sperm Mediator	Main Findings	Key Limitation	Reference
BPA	Zebrafish	Adult paternal preconception exposure: males exposed to BPA (100 or 2000 µg/L) for 2 or 3 weeks during defined spermatogenic windows before mating with untreated females	F1	Early embryonic abnormalities, increased apoptosis and mortality; severe exposure caused marked developmental failure	Sperm H3K27ac/H3K9ac alterations, with concurrent sperm DNA fragmentation	BPA altered sperm histone acetylation, with related hyperacetylation patterns detected in F1 embryos; developmental toxicity was particularly severe after exposure encompassing whole spermatogenesis	Only F1 examined; zebrafish model and relatively high exposure; histone-mediated programming cannot be separated from concurrent sperm DNA damage; no direct functional test of the candidate sperm alteration was performed	[71]
BPA	Zebrafish	Adult paternal preconception exposure: males exposed during spermatogenesis before fertilization of eggs from untreated females	F1	Abnormal cardiogenesis/cardiac phenotype in progeny	Sperm/embryonic histone hyperacetylation, including H3K9ac; altered regulation of *kat6a*, *esr2b* and *hand2*	Paternal BPA altered sperm epigenetic state and embryonic cardiogenic programs; EGCG intervention normalized histone acetylation/gene expression and ameliorated the cardiac phenotype, providing functional rescue evidence supporting the proposed pathway	F1 only; fish model; intervention supports involvement of histone acetylation but does not establish a single sperm-borne causal mark	[109]

Table 2. *Cont.*

Exposure	Study/Model	Exposure Scenario and Window	Generation	Developmental/Offspring Outcome	Candidate Sperm Mediator	Main Findings	Key Limitation	Reference
BPA	Mouse	Adult paternal preconception exposure: F0 males exposed to BPA (~50 µg/kg/day for 21 weeks) before mating with healthy females	F1	Early leptin resistance followed by increased adiposity, dyslipidemia, impaired glucose tolerance/insulin sensitivity and hepatic metabolic abnormalities	Sperm miR-149-5p, miR-150-5p and miR-700-5p via PPARγ–Dicer1 signaling	BPA altered spermatogenic miRNA biogenesis; the sperm miRNA signature converged on offspring hepatic Lepr signaling. Zygotic delivery of the candidate miRNAs reproduced suppression of the Lepr axis, providing functional evidence for a contribution of the candidate sperm miRNAs to the proposed pathway	F1 only; exposed fathers themselves developed metabolic abnormalities, complicating separation of direct BPA germline effects from programming secondary to paternal metabolic state	[144]
BPA	Rare minnow	Adult paternal preconception exposure: F0 males exposed to BPA (15 µg/L) for 14 days and mated with untreated females; F1 males subsequently bred with untreated females	F1–F2	F1 showed increased malformations, reduced mandibular dimensions and delayed craniofacial osteogenesis; phenotype was absent in F2	Altered sperm miRNAs; aca-miR-16a-5p–*bmp2a* proposed	Twenty-eight sperm miRNAs were altered after paternal exposure; F1 craniofacial defects were accompanied by altered osteogenic signaling, whereas F2 showed no corresponding phenotype	Fish model; candidate miRNA pathway is predictive/correlative without sperm-RNA transfer or rescue; no transgenerational persistence	[123]

Table 2. *Cont.*

Exposure	Study/Model	Exposure Scenario and Window	Generation	Developmental/Offspring Outcome	Candidate Sperm Mediator	Main Findings	Key Limitation	Reference
BPA/BPF	Prospective human ART cohort (n = 128 men)	Human paternal preconception exposure: urinary BPA and BPF measured before ART	Embryo/pregnancy/F1 birth outcome	Lower fertilization and live-birth probability with higher paternal BPA exposure	Not measured	Highest paternal BPA quartile was associated with lower fertilization (β = −0.10, 95% CI −0.18 to −0.02) and lower live birth (RR = 0.27, 95% CI 0.09–0.86); mixture analysis also indicated lower live birth, predominantly driven by BPA	Observational infertility-clinic cohort; relatively small sample; spot urine measurement; no sperm molecular mediator; BPF alone was not significantly associated with live birth	[145]
BPS	Rat	Adult paternal preconception exposure: F0 males received BPS (4 or 40 µg/kg/day) for 2 months before mating with untreated females	F1	Male offspring developed increased adiposity, reduced insulin sensitivity, hyperinsulinemia and later glucose intolerance, particularly at the lower dose	Not measured	Preconception paternal BPS produced sex-specific and non-monotonic metabolic abnormalities in male offspring, including impaired muscle insulin signaling and increased β-cell mass	F1 only; no sperm epigenetic profiling or functional transmission experiment; therefore mechanism of paternal transmission remains unresolved	[146]

Table 2. *Cont.*

Exposure	Study/Model	Exposure Scenario and Window	Generation	Developmental/Offspring Outcome	Candidate Sperm Mediator	Main Findings	Key Limitation	Reference
DEHP	Mouse	Adult paternal preconception exposure: males exposed to DEHP (2 or 10 mg/kg/day) before mating with unexposed females	F1 embryo/extraembryonic tissues	Altered embryonic and extraembryonic DNA methylation and transcriptional programming	Sperm DNA methylation/DMRs	Paternal DEHP generated 704 sperm DMRs; 29 sperm DMRs overlapped DMRs identified in F1 embryonic or extraembryonic tissues, supporting molecular continuity between the paternal germline and early development	DMR overlap does not establish causal transmission; no targeted epigenetic manipulation/transfer; endpoints primarily represent early molecular programming rather than later offspring disease	[105]
DCHP	Mouse	Adult paternal preconception exposure: F0 males exposed to DCHP, followed by breeding with unexposed females and paternal-line follow-up	F1–F2	Metabolic dysfunction in descendants, including disturbances in glucose/lipid homeostasis	Altered sperm small non-coding RNAs	Paternal DCHP exposure altered the sperm small-RNA landscape and was associated with metabolic abnormalities extending from F1 into F2, supporting a germline-linked mechanism	Sperm-RNA changes are associated with descendant phenotypes but were not established as the sole causal mediator by purified RNA transfer/rescue; F2 provides evidence of a potential transgenerational effect	[114]

Table 2. *Cont.*

Exposure	Study/Model	Exposure Scenario and Window	Generation	Developmental/Offspring Outcome	Candidate Sperm Mediator	Main Findings	Key Limitation	Reference
DEHP	Mouse	Adult paternal preconception exposure: DEHP exposure before conception followed by mating with untreated females	F1	Disturbed offspring glucose metabolism/glycometabolic homeostasis	Sperm miR-10a-5p	Paternal DEHP altered sperm miRNA composition, with miR-10a-5p implicated in the metabolic programming observed in offspring	Predominantly F1 evidence; generalizability to environmentally encountered human exposures remains uncertain	[127]
Phthalate mixtures	Prospective human fertility/ART cohort	Human paternal preconception exposure: urinary phthalate metabolites and BPA assessed before fertility treatment	Pregnancy/live birth	Reduced probability of achieving live birth for selected paternal exposure mixtures	Not measured	Higher paternal DEHP-related exposure mixture was associated with a greater probability of failure to achieve live birth (HR ≈ 1.41, 95% CI 1.08–1.81); findings support a paternal contribution to reproductive success beyond maternal exposure	Observational ART population; urinary biomarkers have short biological half-lives; residual confounding; no sperm molecular mediator and no inference of epigenetic transmission	[135]

Table 2. *Cont.*

Exposure	Study/Model	Exposure Scenario and Window	Generation	Developmental/Offspring Outcome	Candidate Sperm Mediator	Main Findings	Key Limitation	Reference
Micro-/nanoplastics	Mouse	Adult paternal preconception exposure: nano(micro)plastic exposure before conception, followed by mating with unexposed females	F1	Sex-dependent offspring metabolic abnormalities	Sperm small ncRNAs, particularly tsRNA/rsRNA signatures	PANDORA-seq identified exposure-associated remodeling of sperm small ncRNAs; offspring displayed sex-specific metabolic phenotypes, with complementary cellular experiments supporting biological activity of candidate RNA signals	F1 only; altered sperm RNAs are strong candidate mediators but direct causal transmission of the complete offspring phenotype was not definitively demonstrated	[65]
Polystyrene nanoplastics	Mouse	Adult paternal preconception exposure: PS-NP exposure before conception followed by unexposed breeding across generations	F1–F2	BPD-like pulmonary injury and altered lung development, with ferroptosis-related abnormalities persisting into F2	Sperm FtMt promoter hypermethylation, associated with DNMT3A	Paternal PS-NPs induced sperm FtMt hypermethylation and pulmonary abnormalities in F1 and F2; IVF/embryo-transfer and DNMT3A-targeted experiments provided functional evidence supporting a sperm-originating epigenetic mechanism	Experimental mouse model; locus-specific mechanism requires validation across exposure conditions and in humans. F1 is intergenerational; F2 is the first eligible transgenerational generation after adult paternal exposure	[66]

Table 2. *Cont.*

Exposure	Study/Model	Exposure Scenario and Window	Generation	Developmental/Offspring Outcome	Candidate Sperm Mediator	Main Findings	Key Limitation	Reference
Polystyrene nanoplastics	Human sperm, in vitro	Direct in vitro sperm exposure: human sperm exposed to PS-NPs under experimental conditions	Not applicable	Reduced sperm functional competence; no offspring outcome assessed	Oxidative/mitochondrial injury and DNA damage; no specific epigenetic mediator demonstrated	PS-NP exposure impaired human sperm function and was associated with oxidative and mitochondrial dysfunction	In vitro exposure does not reproduce an in vivo paternal exposure; no fertilization, embryo, or offspring assessment; no evidence of germline-mediated transmission	[51]
Polystyrene nanoplastics	Mouse	Adult paternal preconception exposure: male mice received 500 nm PS-NPs (50 mg/kg/day) orally for 35 days; PS-NP + NMN rescue group included; sperm used for IVF	F1 early embryos	Reduced fertilization capacity, extensive transcriptomic dysregulation in 2-cell embryos, and increased embryonic ROS; NMN ameliorated these abnormalities	No specific epigenetic mediator demonstrated; sperm oxidative/mitochondrial injury and DNA damage implicated	PS-NPs impaired paternal sperm function and produced early embryonic toxicity after IVF; 2417 embryonic DEGs were identified, while NMN partially restored fertilization, embryonic redox state, and transcriptional profiles	High experimental exposure; developmental follow-up limited to early embryos; sperm small RNAs/DNA methylation were not profiled, and direct evidence of epigenetic transmission is lacking	[51]

6.6. Overall Interpretation

However, experimental and human studies provide evidence that paternal plastic-associated exposures may be associated with altered embryonic development, pregnancy outcomes, and offspring phenotypes [65,147]. The evidentiary significance of molecular alterations, intermediate reproductive endpoints, and clinically relevant phenotypes varies according to the exposure investigated, the experimental design, and the outcomes assessed.

A major unresolved question is the extent to which molecular alterations in sperm directly contribute to developmental outcomes. The co-occurrence of paternal exposure, sperm molecular alterations, and offspring phenotypes alone is insufficient to establish causality; evidence demonstrating a mechanistic link between these events is also required [101,148]. This question will be explored in the following section.

7. Intergenerational and Transgenerational Effects

The potential effects of plastic-associated exposures across generations have attracted considerable interest, but their interpretation requires careful distinction between directly exposed and unexposed generations [149]. Effects observed in offspring do not necessarily indicate generational inheritance, and persistence across generations does not by itself establish an epigenetic mechanism. Classification of generations would have to be determined by exposure and breeding [150].

7.1. Exposure Windows and Generational Definitions

When an adult male is exposed before conception, both the F0 male and the sperm that give rise to the F1 generation are directly exposed. The effects noted in F1 progeny would therefore be considered intergenerational. If the exposure has stopped and future generations are not re-exposed, then F2 is the first generation that could be tested for a transgenerational effect [17,148]. This distinction identifies the generation in which the phenotype occurs but does not establish the mechanism underlying the observed effect.

The interpretation differs when paternal exposure occurs during the father's own prenatal development. The treatment of the F0 female while pregnant will expose the developing F1 offspring and the germ cells that will eventually produce the F2 offspring. In such a case, F3 would usually be the first generation without direct exposure [17,148]. This classification is unchanged when transmission occurs through the F1 male, as the F2 generation remains intergenerational following gestational exposure of the F0 female, regardless of the paternal line of transmission. Research in which the father was exposed during fetal development should be differentiated from research in which the father was exposed as an adult [151]. Direct experimental exposure of isolated sperm, germ cells, or testicular cells represents a further distinct scenario. Such studies can provide mechanistic information on cellular responses to an exposure but do not reproduce an in vivo paternal exposure or, in the absence of subsequent fertilization and offspring assessment, provide evidence of intergenerational or transgenerational effects.

The term "multigenerational" may describe effects observed across multiple generations without specifying whether they are intergenerational or transgenerational [152]. For the purposes of this review, classification is therefore based on the experimental breeding design and timing of exposure, irrespective of the terminology used in individual studies [153]. Particular attention is given to exposures occurring during gestation, lactation, or before mating, as their timing determines which generations and germ cells are directly exposed [25].

7.2. Evidence from Plastic-Associated Chemical Exposures

Several experimental studies have reported phenotypic effects extending beyond directly exposed individuals through the paternal lineage. In the mouse study discussed in Section 6, adult F0 males were exposed to BPA before mating with unexposed females, with no subsequent BPA exposure in their descendants. Reproductive abnormalities were observed in the F2 generation, while some alterations in sperm fertility and DNA methylation were also detected in F3. Sperm DNA methylation patterns also varied across generations rather than being consistently maintained. Because exposure was restricted to adult F0 males, the phenotypic alterations observed in F2 and F3 are consistent with transgenerational effects [16].

Paternal DCHP exposure provides evidence consistent with a potential transgenerational effect. Following exposure of F0 males to DCHP, subsequent breeding through unexposed F1 males resulted in impaired glucose tolerance in F2 female offspring following a high-fat diet challenge. Because the F2 phenotype occurred in descendants without direct exposure, it is consistent with a transgenerational effect in this experimental model. Although alterations in sperm RNA profiles suggest a potential RNA-mediated mechanism, their causal role in transmitting the phenotype across generations has not been established [114].

Developmental exposure represents a distinct generational exposure paradigm. In rats, gestational exposure of F0 females to a mixture of BPA, DEHP, and dibutyl phthalate was associated with reproductive and metabolic disease phenotypes and altered sperm DNA methylation in the F3 generation. In this exposure model, the F0 female, the developing F1 offspring, and the F1 germ cells that will give rise to the F2 generation are directly exposed; consequently, F3 represents the first generation without direct exposure. The findings therefore provide evidence of transgenerational effects following ancestral developmental exposure rather than adult paternal preconception exposure. However, because the study used a chemical mixture, the observed effects cannot be attributed to any individual component [154].

7.3. Persistence of Phenotypes and Epigenetic Information

Persistence of a phenotype across generations does not necessarily require complete conservation of the associated molecular signature. As discussed in Section 5, extensive epigenetic reprogramming occurs after fertilization and during germ cell development [117,118]. Any proposed mechanism of transgenerational transmission must therefore account for how exposure-related information either persists despite this reprogramming or contributes to the re-establishment of a regulatory state in subsequent generations.

Molecular alterations detected in the sperm or somatic tissues of subsequent generations may arise through several mechanisms [155]. A molecular signal may persist at specific loci, emerge secondarily as a consequence of altered physiology in the descendant, or act transiently during early development to initiate regulatory processes that subsequently re-establish a similar molecular state [18]. Therefore, detection of a similar molecular state in descendant sperm does not by itself demonstrate that the original exposure-induced signal was directly transmitted from the exposed ancestor.

Phenotypic persistence and molecular persistence should therefore be evaluated independently. Detection of a molecular alteration in a later generation does not demonstrate persistence of the original molecular mechanism, just as recurrence of a phenotype does not by itself identify the mechanism of transmission. Establishing a causal link between the two requires direct mechanistic evidence [18,148].

7.4. Requirements for Establishing Germline-Mediated Inheritance

Demonstrating transgenerational effects requires an experimental design that clearly identifies the first generation without direct exposure, confirms cessation of the exposure, and minimizes the possibility of residual exposure or re-exposure [148,156]. Establishing germline-mediated inheritance requires additional mechanistic evidence. Mating exposed males with unexposed females can strengthen the attribution of observed effects to the paternal lineage; however, natural mating alone cannot exclude contributions from paternal factors other than sperm [20].

Establishing mechanistic causality requires additional evidence. When accompanied by appropriate controls, assisted fertilization and embryo transfer approaches can help determine whether observed effects are mediated through sperm rather than other paternal or maternal responses to mating. Functional manipulation of candidate sperm-borne components, including targeted inhibition, transfer, or rescue experiments, can provide stronger evidence that a specific molecular alteration contributes to the resulting phenotype. Distinguishing genetic from epigenetic mechanisms is also essential when interpreting potential germline-mediated effects [20,115,148].

Confirming cessation of exposure is particularly important for plastic particles, as persistence of particles within the organism or inadvertent continued exposure could complicate the interpretation of effects across generations. Following adult paternal exposure, an F1 phenotype, such as that reported after polystyrene microplastic exposure in mice, is consistent with an intergenerational effect [65]. Demonstration of a transgenerational effect would require phenotypic persistence in the first and/or subsequent generations not directly exposed.

7.5. Human Relevance

The human studies of paternal exposure, reproductive outcomes, and first-generation offspring included in this review do not establish transgenerational epigenetic inheritance. Demonstrating transgenerational effects in humans would require multigenerational evidence capable of distinguishing biological inheritance from repeated exposure and shared environmental, socioeconomic, lifestyle, and genetic factors. Human observational studies are also limited by potential residual confounding and by exposure misclassification, particularly when biomarkers reflect relatively short exposure windows. This distinction is particularly challenging for plastic-associated exposures because their widespread and recurrent nature makes it difficult to identify a truly unexposed generation of descendants [133,148].

Experimental animal and in vitro studies provide greater control over exposure conditions and can support mechanistic investigation, but differences in exposure levels, routes, duration, experimental conditions, and biological context limit direct extrapolation to human reproductive health. Importantly, associations between paternal exposure, sperm molecular alterations, and offspring outcomes in humans do not establish a causal sequence linking these events.

Current evidence provides varying levels of support for intergenerational developmental effects, transgenerational phenotypic transmission in specific experimental models, and candidate germline-mediated mechanisms. However, the available evidence is insufficient to establish causal paternal epigenetic transmission or transgenerational inheritance in humans. Maintaining clear distinctions among these levels of evidence is essential for interpreting the implications of paternal exposure without drawing conclusions beyond those supported by the available data.

8. Human Relevance and Methodological Limitations

Interpretation of studies examining paternal exposure in relation to human reproduction and offspring health should consider how closely experimental exposures reflect human exposure, as well as study design and the strength of causal inference. The studies discussed above identify potential mechanisms and developmental effects associated with paternal exposure to reproductive toxicants. However, in humans, a causal link between plastic-associated exposures, molecular alterations in sperm, and subsequent adverse health outcomes in offspring has not been established [131,133]. Several factors limit the synthesis and translation of the available evidence.

8.1. Exposure Relevance and Biological Dose

Experimental studies frequently use standardized plastic particles or defined chemicals administered at controlled doses. Although such designs facilitate mechanistic investigation, they do not fully reflect human exposure patterns, which typically involve complex chemical mixtures and plastic particles with heterogeneous sizes and physicochemical properties. The route of exposure is also an important consideration when interpreting experimental findings. For example, polystyrene particles were administered by oral gavage in the mouse study discussed above [65].

Accordingly, claims of "environmentally relevant" exposure should be evaluated against empirical measurements of human exposure. Similarity between an experimentally administered dose and an estimated level of human intake does not necessarily imply comparable internal exposure or equivalent concentrations within the testes or sperm. Whenever feasible, experimental studies should relate external dose to internal exposure measurements, including concentrations in reproductive tissues and biological samples [157]. This consideration is particularly important for particle-based exposures, for which dose may be expressed using different metrics, including polymer mass, particle number, and surface area.

The timing of exposure assessment represents an additional challenge in human biomonitoring studies. Urinary biomarkers of BPA and phthalate exposure primarily reflect recent exposure and may therefore inadequately characterize exposure over earlier stages of spermatogenesis and sperm maturation. Repeated-sampling studies have demonstrated substantial within-person temporal variability in urinary BPA and phthalate metabolite concentrations [158,159].

8.2. Particle Characterization and Analytical Comparability

Research on plastic particles requires characterization beyond their classification as "microplastics" or "nanoplastics." Particle size and size distribution, shape, surface properties, aggregation state, and interactions with associated chemicals may influence biological activity and the interpretation of experimental findings [29,160]. Consequently, results obtained using uniformly sized polystyrene particles should be interpreted as specific to the material and particle characteristics tested and should not be generalized to other plastic types without supporting evidence.

Differences in human biomonitoring methods may also limit comparability across studies. Pyrolysis-based approaches quantify polymer mass, whereas spectroscopic techniques detect and characterize individual particles within method-specific size and detection limits. Polymer mass cannot be directly converted to particle number without assumptions regarding particle size, morphology, and composition [48,161].

Rigorous contamination control is essential because collection materials, laboratory equipment, solvents, and airborne particles may introduce plastic-associated signals. Where applicable, experimental studies should report procedural blanks, recovery assessments,

identification criteria, and limits of detection [162]. Detection within a biological sample should also be clearly distinguished from localization within a specific tissue or cellular compartment [48]. Detection in semen does not establish localization within spermatozoa, while detection in testicular tissue does not necessarily demonstrate direct exposure of germ cells [45].

8.3. Confounding and Selection in Human Studies

Paternal and maternal exposures tend to be associated since partners share their food, housing, consumer goods, and other environmental exposures. Associations involving paternal biomarkers may also be influenced by maternal exposures or shared environmental and lifestyle factors that independently affect pregnancy and offspring health. For example, adjustment for maternal preconception DEHP exposure attenuated the association with preterm birth. Assessing exposure in both partners is particularly informative, although residual confounding may persist because of biomarker measurement error and unmeasured exposures [137,138].

Additional potential confounders and sources of variability include parental age, smoking, adiposity, dietary habits, occupational exposures, socioeconomic status, and reproductive history. Recruitment through fertility clinics would need to take into account the selection issue since the subjects selected may be different from the general population in terms of their reproductive capability, infertility diagnosis, and treatments received. The results from assisted reproduction are thus useful but should not be automatically applied to natural conception [136].

Pregnancy and childhood follow-up introduce additional opportunities for selection bias. Studies restricted to live-born children necessarily exclude pregnancies that do not result in live birth, which may influence observed associations if the exposure under investigation also affects pregnancy survival [163]. Incomplete follow-up may further introduce selection bias if families retained in the study differ systematically from those lost to follow-up, potentially influencing observed associations between preconception exposures and subsequent outcomes [164].

8.4. Molecular Endpoints and Causal Attribution

In studies of environmental effects on reproduction, molecular alterations in sperm may serve as biomarkers of exposure, correlates of reproductive dysfunction, or potential mediators of developmental outcomes. Appropriate study designs and functional validation are required to determine the biological significance and potential causal role of these molecular alterations.

Persistence of identical molecular alterations across sperm and descendant tissues should not be assumed, as illustrated by the limited correspondence between sperm and embryonic tissue DNA methylation changes following paternal DEHP exposure [105]. Similarly, altered sperm small RNA profiles following paternal polystyrene exposure, together with the regulatory effects of selected RNA species in embryonic stem cell models, provide mechanistic insight but do not establish their causal role in generating the observed offspring phenotype [65]. Stronger evidence for causal mediation would require functional approaches such as transfer, depletion, rescue, or targeted manipulation of the candidate molecule.

The molecular profiles detected in sperm are also influenced by technical and analytical factors. Transparent reporting of sperm sample quality, somatic cell contamination, sequencing depth, batch effects, and statistical criteria is therefore essential. Conventional bisulfite sequencing cannot distinguish 5-methylcytosine from 5-hydroxymethylcytosine [165], while certain modified RNA species may be underrepresented by standard small RNA library preparation methods [166]. The additional small RNA species revealed using PANDORA-seq in the DCHP study illustrate how analytical methodology can influence the molecular profiles that are detected [114]. Accordingly, replication across independent studies, methodological validation, and assessment of biological relevance should precede the clinical development of candidate biomarkers.

8.5. Experimental Design and Outcome Interpretation

Animal experiments should account for both the independently exposed sire and the hierarchical organization of offspring within each litter. Increasing the number of offspring examined from a limited number of families does not provide the same level of replication as increasing the number of independent exposures. The reliability of studies assessing multiple endpoints can be strengthened through randomization, blinded outcome assessment, prespecified analyses, and transparent reporting of both positive and null findings [167].

The observed outcomes may also vary according to offspring sex, age at assessment, and postnatal environmental factors. The mouse study of polystyrene particle exposure assessed metabolic outcomes following a high-fat diet challenge [65]. Although such experimental designs may demonstrate susceptibility under specific conditions, they do not establish that the same phenotype occurs under unchallenged or naturally occurring conditions. Overall, molecular alterations, spermatogenic defects, embryonic phenotypes, questionnaire-based behavioral measures, and clinical conditions represent different levels of evidence and should not be considered equivalent endpoints.

These considerations do not diminish the value of the experimental or observational findings but define the limits of the conclusions that can be drawn [168]. Stronger human evidence would require repeated assessment of parental exposures combined with molecular analyses of sperm and longitudinal follow-up of reproductive and offspring outcomes. Such studies could subsequently inform targeted experimental investigations of specific candidate pathways [133].

9. Research Priorities and Preconception Implications

Future research should determine which paternal exposures influence reproductive and offspring outcomes, elucidate the underlying mechanisms, and assess whether reducing exposure can mitigate these effects [38,131,133]. Addressing these questions will require integrating evidence from human and experimental studies that link the timing and level of exposure to molecular alterations in sperm, reproductive outcomes, and developmental outcomes [98,131,133], as summarized in Figure 2.

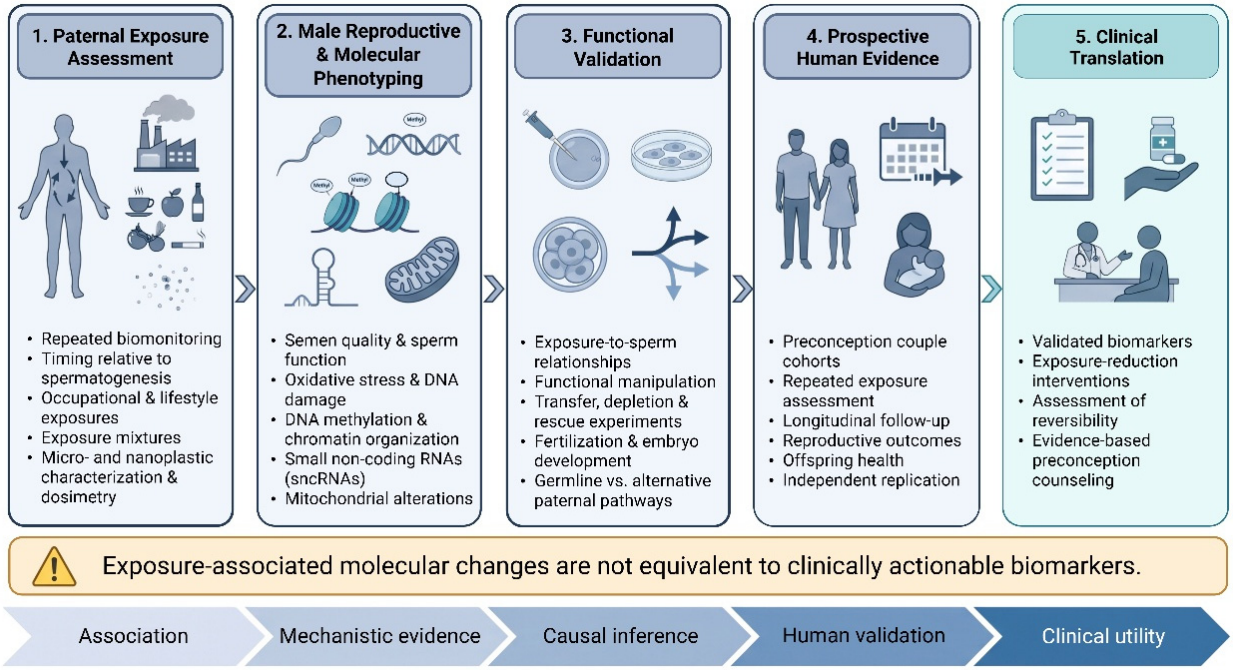


Figure 2. Framework for advancing research on paternal exposure to plastic-associated pollutants toward clinical translation. Created in BioRender. Potiris, A. (2026) https://BioRender.com/8kadhas.

9.1. Prospective Studies of the Paternal Preconception Window

Recruiting both partners before conception and performing repeated exposure assessments during biologically relevant periods of spermatogenesis and post-testicular sperm maturation present important methodological challenges [72–74,158,159]. Longitudinal follow-up should include time to pregnancy, pregnancy loss, birth outcomes, and, where feasible, offspring health and development [130,131,133]. Studies should also include couples conceiving without assisted reproductive technologies to extend the evidence beyond populations undergoing fertility treatment [134,135,138,145].

Concurrent assessment of maternal exposures may help distinguish paternal-specific associations from those arising from shared exposures or maternal influences [134,138]. Exposure assessment should integrate biomarker measurements with detailed information on occupational history, diet, residential factors, and product use. A paternal exposome framework may be particularly valuable for characterizing multiple concurrent exposures, provided that comprehensive exposure profiling is hypothesis-driven and supported by adequate statistical power [130,131,133].

Distinguishing exposures occurring during the father's own development from those occurring during adulthood is also essential [98,131]. Longitudinal studies incorporating repeated exposure assessments and serial semen collection may help characterize adult exposure windows and their temporal relationship with changes in sperm characteristics [72–74,158,159].

9.2. Mechanistic Validation of Candidate Sperm Signals

A major research priority is to determine whether exposure-associated molecular alterations in sperm contribute to subsequent developmental outcomes [5,101,133]. Integrated analyses of DNA methylation, chromatin organization, and small RNA profiles may identify coordinated molecular pathways, but they cannot establish causal mediation on their own [1,18,101]. Experimental studies should therefore evaluate the contribution of specific sperm-borne factors through approaches such as transfer, depletion, targeted manipulation, or rescue of the phenotype of interest [4,19,20,115].

The design of the experiment must match the mechanism. DNA methylation studies should characterize the functional relevance and fate of candidate regions after fertilization [101,102], chromatin studies should distinguish histone retention from post-translational modifications [1,101,108], and RNA studies should use physiologically relevant amounts of candidate molecules.

Mechanistic studies should also distinguish sperm epigenetic alterations from alternative pathways through which paternal exposures may influence reproductive and developmental outcomes, including DNA damage, seminal plasma-mediated effects, and broader alterations in paternal physiology [1,98,131,132]. Molecular characterization combined with functional validation will be necessary to determine the relative contribution of these processes [18,101,115].

9.3. Standardization of Particle and Mixture Research

Greater standardization of particle characterization, dosimetry, sample preparation, contamination control, and analytical reporting is needed to improve comparability and distinguish the effects of polymer type, particle properties, weathering, and associated chemicals [29,48,55,157,160–162].

Experimental studies should increasingly incorporate environmentally relevant and heterogeneous materials rather than relying solely on standardized laboratory particles, while using appropriate controls to separate the effects of the particles themselves from those of associated or released chemicals [30,67,68,160]. This is essential in order to identify which properties of the particles are important for reproductive toxicity [27,55].

This also applies to studies of environmental mixtures, which should balance exposure realism with mechanistic interpretability. Realistic mixtures should be investigated alongside their individual components to distinguish chemical-specific effects from additive or interactive effects [135,138]. Human studies should incorporate adequately powered statistical designs, appropriate approaches for mixture analysis, and independent validation where feasible [133,168].

9.4. Reversibility and Exposure Reduction

The reversibility of paternal reproductive and molecular effects will be particularly important to establish in preconception research [98,130,131]. Experimental studies should include post-exposure follow-up to determine whether sperm function, molecular profiles, and developmental outcomes recover over time. Importantly, normalization of a sperm biomarker does not necessarily indicate complete biological recovery.

Human intervention studies could evaluate practical exposure-reduction strategies, with biomonitoring used to confirm changes in exposure levels [38]. Outcomes should distinguish between reduced exposure, improved semen quality, reproductive success, and offspring health, as these represent different levels of evidence [38,130,131].

Potential substitution effects should therefore be considered when evaluating the effectiveness of an intervention. Exposure to replacement chemicals and materials should also be evaluated, as substitution may alter rather than reduce the overall exposure mixture [8,33,134]. Ultimately, research should inform practical strategies to reduce exposure and, where possible, improve reproductive outcomes [38,130].

9.5. Preconception Communication and Clinical Translation

Although paternal environmental exposures should be considered in preconception research and counseling, clinical recommendations should remain proportionate to the strength of the available evidence [130–133]. It is important to determine whether occupa-

tional safety measures, environmental exposure education, and other interventions that improve reproductive outcomes also reduce adverse outcomes in offspring.

Molecular signatures associated with paternal environmental exposures have been identified in human sperm [7,14,15,103,104], but development of a clinically applicable biomarker will require reproducible analysis, independent validation, demonstrated predictive value beyond standard reproductive assessment, and evidence that the resulting information can guide clinical decisions.

Communication about paternal exposures during preconception counseling should avoid deterministic interpretations [130–133]. Experimental evidence of susceptibility does not imply that an exposure will necessarily affect offspring health, and prevention should not rely solely on individual responsibility. Personal exposure-reduction strategies should be complemented by safer product design, occupational protections, and broader efforts to reduce environmental contamination. Ultimately, determining whether and when exposure reduction improves sperm quality and reproductive outcomes remains a key research priority [38,130].

10. Conclusions

The relevance of plastic-associated pollutants to paternal reproductive toxicology extends beyond conventional semen parameters to their potential effects on the molecular features of sperm. Human studies provide evidence linking paternal exposures with reproductive outcomes and alterations in sperm DNA methylation, chromatin, and non-coding RNAs, as well as OS, mitochondrial dysfunction, endocrine disruption, and changes in the testicular microenvironment. However, there is a fundamental difference between these two groups of plastic pollutants.

Other experimental studies selected here also indicate that paternal exposure is associated with developmental, reproductive, metabolic, and neurobehavioral changes in offspring. However, exposure-related alterations in sperm and phenotypic changes in offspring do not, on their own, establish germline-mediated inheritance. Intergenerational effects should be distinguished from phenotypic persistence across generations, and experimental findings in generations beyond direct exposure do not establish transgenerational epigenetic inheritance in humans.

Future progress in this field will depend on prospective couple studies with repeated exposure assessment, greater standardization of particle research, and functional validation of candidate mediators of male fertility. Determining whether male reproductive effects are reversible and whether reducing exposure improves reproductive outcomes is also essential. Including paternal environmental exposures in preconception research could improve understanding of biologically and clinically relevant exposures, their mechanisms of action, and approaches to prevent or reverse their effects.

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