Environmentally Induced Epigenetic Transgenerational Inheritance and Male Reproductive Pathology

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Abstract

Epigenetic processes allow organisms to respond to their environment with changes in gene expression. Epigenetic molecular processes include DNA methylation, histone modifications, non-coding RNAs, RNA methylation and chromatin structure. Epigenetic information and epimutations can be transmitted to subsequent generations through the germline sperm or eggs, and can change gene expression and an organism's phenotype even when transgenerational generations are themselves never exposed to an inducing factor. All cell types and tissues derived from germ cells carrying epimutations will have the potential for a cell specific altered epigenome and altered gene expression. Male reproductive tissues sensitive to an altered epigenetics and gene expression profile will have a different phenotype and have susceptibility to develop disease or abnormalities as the individual ages. Environmental exposures in past generations will contribute to the incidence of male reproductive abnormalities and disease in the current human population, as well as all organisms.

Glossary

Epigenetic Transgenerational Inheritance Germline (sperm or egg) transmission of epigenetic information between generations in the absence of any continued direct exposures or genetic manipulations.

Epigenetics Molecular factors/processes around DNA that regulate genome activity independent of DNA sequence; and that are mitotically stable.

Key Points

- Environmentally induced epigenetic transgenerational inheritance and male reproductive pathology.

Introduction

Epigenetic processes are the primary mechanisms that organisms use to respond to their environment with changes in gene expression. In addition, it is primarily by epigenetic mechanisms that stem cells are able to differentiate and change to develop into a specific cell type. Epigenetics is defined as: "Molecular factors/processes around the DNA that regulate genome activity independent of DNA sequence, and that are mitotically stable" (Skinner, 2011). There are a variety of epigenetic factors that act around the DNA in a cell to regulate gene expression.

In the 1970's DNA methylation was the first epigenetic molecular mark to be characterized. With DNA methylation a small (methyl) chemical group is attached to DNA, primarily at the cytosine base in animals (Holliday and Pugh, 1975; Riggs, 1975; Singer *et al.*, 1979a,b) to produce 5-methylcytosine (5mC). Other chemical modifications of cytosine bases in DNA have since been described. In broad terms, the presence of 5mC often represses DNA transcription. The functions of the other epigenetic modifications to cytosine are under investigation. N(6)-methyladenine is a less frequent epigenetic modification to the adenine base of DNA that was once thought to only be present in prokaryotic organisms, but has now been described in mammalian embryonic stem cells (Wu *et al.*, 2016).

The histone proteins that DNA is wrapped around create the nucleosome and can be chemically modified to alter gene expression. There are many different histone post-translational modifications including lysine acetylation, lysine and arginine methylation, arginine citrullination, lysine ubiquitination, lysine sumoylation, ADP-ribosylation, proline isomerization, and serine/threonine/tyrosine phosphorylation (Rothbart and Strahl, 2014). These modifications can change chromatin structure or recruit transcriptional cofactors to DNA to regulate gene expression.

Non-coding RNA molecules can act as epigenetic factors (Kornfeld and Bruning, 2014). These are small RNA molecules that do not code for a protein, but rather function as RNA to regulate gene expression. The non-coding RNA molecules that act as epigenetic factors are not DNA sequence dependent, so the majority do not depend on having a nucleotide sequence that is complimentary to a specific DNA or RNA region in order to function. Long non-coding RNAs (lncRNAs) (Wei *et al.*, 2017) and transfer RNA-derived small RNAs (tsRNAs) (Chen *et al.*, 2016a) are examples of RNA classes that are present in sperm and eggs as epigenetic factors that affect subsequent generations (Chen *et al.*, 2016b). The ncRNA can mediate epigenetic transgenerational inheritance (Schuster *et al.*, 2016).

The coiling, looping and general structure of DNA, termed chromatin structure, is also an epigenetic factor (Yaniv, 2014). The three-dimensional structure of DNA can make certain regions of the genome accessible to transcription machinery, or bring enhancer regions near to gene promoters, and so affect gene expression. Therefore, epigenetic molecular processes include DNA methylation, histone modifications, non-coding RNAs, RNA methylation and chromatin structure.

Altered epigenetic marks at specific DNA sites in response to exposure to an environmental factor are termed 'epimutations' (Skinner *et al.*, 2010). Thus, DNA sequence changes are genetic mutations, while environmentally altered epigenetic sites that influence genome activity are epimutations.

Epigenetic Transgenerational Inheritance

Epigenetic information can be transmitted to subsequent generations through the germline, and can change gene expression and an organism's phenotype in transgenerational generations that were themselves never exposed to an inducing factor like an environmental toxicant. The ability to transmit epigenetic information from one generation to the next requires that alteration in epigenetic factors be present in sperm or eggs. If the sperm or egg cells are transmitting epigenetic information transgenerationally then epimutations should be detectable in these germ cells. Analysis of the F3 generation (great-grand-offspring) sperm from a line of rats in which the F0 generation gestating pregnant females were treated with the environmental toxicant vinclozolin were found to have sperm epimutations consisting of altered DNA methylation, compared to controls (Anway *et al.*, 2005; Guerrero-Bosagna *et al.*, 2010). Interestingly, a variety of different environmental toxicants, each shown to promote transgenerational disease, were found to promote a unique signature or pattern of epimutations in their F3 generation sperm (Ben Maamar *et al.*, 2021c; Manikkam *et al.*, 2012a; Nilsson *et al.*, 2022; Skinner and Nilsson, 2021).

The example of the processes involved in environmentally induced epigenetic transgenerational inheritance is presented in **Fig. 1**. The exposure of an F0 generation gestating female to an environmental factor at the critical window of gonadal (testis or ovary) sex

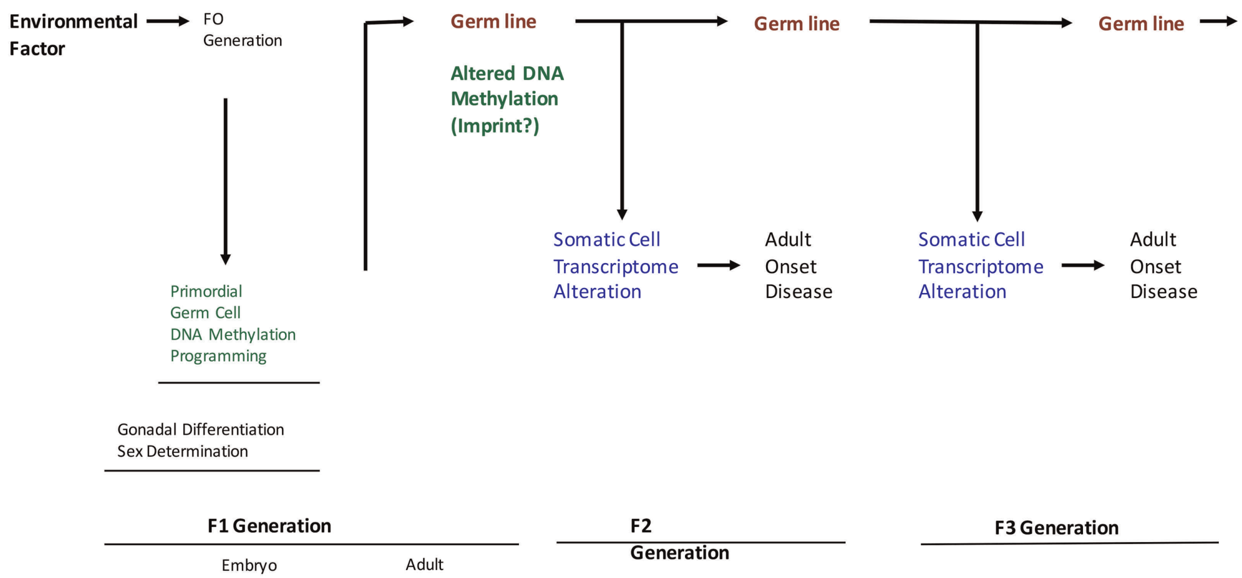

Fig. 1 Role of germline in epigenetic transgenerational inheritance. Summary of environmentally induced epigenetic reprogramming of primordial germ cells that leads to the germline transmission of epimutations, resulting in all somatic cells having altered gene expression. This can result in changes in phenotype or increased disease susceptibility in the F1, F2 or transgenerational F3 generation. Modified from Skinner *et al.* (2010).

determination in the F1 generation fetus modifies the epigenetic programming of the germ cell that the F1 generation adult animal will transmit to the F2 generation. However, any exposure of a male or female during development also has the capacity to directly alter the germline epigenetics to induce epigenetic transgenerational inheritance. All cell types and tissues derived from germ cells carrying epimutations will have an altered epigenome and potentially have altered gene expression. For example, isolated transgenerational cells such as Sertoli cells from the testis or granulosa cells from the ovary have been shown to have a transgenerational alteration in gene expression and epigenetics (Guerrero-Bosagna *et al.*, 2013; Nilsson *et al.*, 2012). Tissues sensitive to an altered epigenetics and gene expression profile will have a different phenotype, and may develop disease or abnormalities as the individual ages. For example, the testis and ovary develop pathologies that are associated with the somatic cell epigenetic alterations (Guerrero-Bosagna *et al.*, 2013; Nilsson *et al.*, 2012). An adult F2 generation individual will then transmit germ cell epimutations to the transgenerational F3 generation, and the same mechanism and process can occur in all subsequent generations, **Fig. 1**.

Environmental exposures can promote non-genetic inheritance through epigenetic transgenerational inheritance mechanisms to promote disease and altered phenotypes in subsequent generations. The potential role of this mechanism in our understanding of disease etiology needs to be considered. For example, environmental exposures in past generations could contribute to the incidence of abnormalities and disease in the current human population. Epigenetic transgenerational inheritance will also have critical roles in other biological processes such as evolutionary biology (Skinner and Nilsson, 2021; Skinner, 2015).

Epigenetic Transgenerational Impacts on Male Reproductive Pathology

A large number of environmental factors and toxicants have been studied in animal models and humans for the potential epigenetic transgenerational effects they have on male reproductive pathology.

Vinclozolin is a fungicide used in the fruit and vegetable industry, which is an antiandrogen substance (Anway and Skinner, 2006). Following exposure of F0 generation pregnant rats to vinclozolin, the direct exposure F1 generation had some testis disease at 1 year age, but when the F2 and transgenerational F3 generation were obtained, significant rates of male reproductive pathology were observed (Anway *et al.*, 2005; Ben Maamar *et al.*, 2018). Observations found abnormal early developmental impacts on spermatogenesis (Anway *et al.*, 2006). The actions were found to impact the primordial germ cell transcriptome and epigenome in early development (Skinner *et al.*, 2013a) and Sertoli cell epigenetics and function (Sadler-Riggleman *et al.*, 2019). Reproductive behavior for the transgenerational males were also found to be effected (Skinner *et al.*, 2014). The transgenerational males had high levels of prostate disease and pathology (Klukovich *et al.*, 2019). All male reproductive pathologies investigated were found to be impacted by ancestral vinclozolin exposure (**Table 1**).

The pesticides of permethrin, an insecticide used to treat both animals and crops (Wang *et al.*, 2016), and DEET (N,N-Diethyl-3-methylbenzamide) is a common active insect repellent applied topically to skin (Osimitz and Grothaus, 1995). Following exposure of F0 generation gestating rats to a mixture of permethrin and DEET and breeding the F1 generation offspring to the F2 and F3 generations demonstrated transgenerational impacts on testis disease, prostate disease, and pubertal abnormalities (Manikkam *et al.*, 2012b; Thorson *et al.*, 2020), **Table 1**.

Dichlorodiphenyltrichloroethane (DDT) is an environmentally persistent insecticide that was heavily used in the United States and Europe in the 1950's and 1960s, and was banned in the USA in 1972. Exposure to DDT may occur when consuming meat, fish, or dairy products (CDC, 2017). Following the exposure of gestating female rats with DDT, the transgenerational F3 generation rats had significant increase in incidence of testis disease, prostate disease, and pubertal pathology (King *et al.*, 2019). Other pathologies include male kidney disease and male obesity (Skinner *et al.*, 2013b). In addition to a transgenerational

Table 1 Environmental (toxicant) impacts on transgenerational male reproductive pathology

Environmental Toxicant	*Transgenerational Effect*	*References*
Vinclozolin	Testis pathology; spermatogenic cell apoptosis; reduced sperm number; prostate disease; reproductive behavior; male infertility	(Anway *et al.*, 2005; Ben Maamar *et al.*, 2018; Klukovich *et al.*, 2019)
Pesticides (Permethrin and DEET)	Testis pathology; prostate disease; pubertal abnormalities	(Manikkam *et al.*, 2012b; Thorson *et al.*, 2020)
DDT (Dichlorodiphenyltrichloroethane)	Testis pathology; prostate pathology; pubertal abnormalities; obesity	(King *et al.*, 2019; Skinner *et al.*, 2013b)
Methoxychlor	Prostate pathology	(Nilsson *et al.*, 2020)
Plastics (BPA, DEHP, and DBP)	Testis pathology; prostate pathology; pubertal abnormalities; obesity	(Manikkam *et al.*, 2013; Thorson *et al.*, 2021)
Glyphosate	Testis pathology; prostate pathology; weaning weights; obesity	(Kubsad *et al.*, 2019)

alteration in DNA methylation, changes in ncRNA levels and histone retention were observed in the transgenerational DDT lineage sperm (Beck *et al.*, 2021). Transgenerational alterations in sperm and Sertoli cells were associated with the increase in male reproductive pathologies (Sadler-Riggleman *et al.*, 2019). The transgenerational DDT impacted male reproductive pathologies are summarized in **Table 1**.

Methoxychlor was the replacement for DDT that is also an estrogenic compound and insecticide (Aoyama and Chapin, 2014). Methoxychlor was also used to exposed F0 generation gestating females at the period of fetal gonadal sex determination, followed by the development of the F3 generation males to assess impacts on male reproductive pathology (Manikkam *et al.*, 2014). Although there was an increase in the transgenerational disease, the primary male reproductive pathology was prostate pathology and negligible testis pathology (Nilsson *et al.*, 2020; Van Cauwenbergh *et al.*, 2020), **Table 1**.

The plastic compounds Bisphenol-A (BPA), dibutyl phthalate (DBP), and di-2-ethylhexyl phthalate (DEHP) are components of plastic that are environmental toxicants that humans are exposed to on a regular basis Lorz Pm *et al.* (2002). After F0 generation pregnant female rats were exposed to a mixture of BPA, DBP, and DEHP, the F3 transgenerational males were found to have significantly increased testis disease, prostate disease, pubertal abnormalities, and male multiple diseases (Manikkam *et al.*, 2013). Other diseases were male kidney disease and obesity. Sperm epigenetic associations with the male reproductive pathologies were also observed (Thorson *et al.*, 2021). A summary of the male reproductive pathology impacted by the plastics are presented in **Table 1**. Similar observations in humans and other animal models for transgenerational pathologies are observed (He and Yin, 2024; Huang *et al.*, 2022).

Dioxin is an organic pollutant that is a by-product of chemical manufacturing processes as well as a common solvent for organic compounds. An example is it being the solvent for the defoliant 2–4-D in agent orange. Dioxin is a reproductive toxicant and can alter hormone release and cause developmental issues or cancer when direct exposure occurs (Who, 2010). The actions of dioxin used an F0 generation gestating female rat exposure during fetal gonadal sex determination to then obtain the transgenerational F3 generation rat to assess male reproductive pathology (Manikkam *et al.*, 2012c). Although increased transgenerational disease was observed, negligible transgenerational male reproductive pathology was observed compared to the F1 generation and F3 generation female (Manikkam *et al.*, 2012c).

Jet fuel JP8 is a hydrocarbon mixture used for military and jet aircraft (Witzmann *et al.*, 2003). After pregnant rats were exposed to jet fuel, the F1 generation rats were bred to the transgenerational F3 generation to assess male reproductive pathologies and epigenetic modification in sperm (Tracey *et al.*, 2013). Although high levels of F1 generation male reproductive pathology was observed for testis, prostate, and puberty pathology, negligible alterations were found in the transgenerational F3 generation reproductive disease. Transient obesity was observed in males (Tracey *et al.*, 2013).

A major current environmental toxicant exposure involves glyphosate (Roundup, Monsanto), which is used in agriculture extensively and yard and lawn use (Bukowska *et al.*, 2022; Milesi *et al.*, 2021; Rossetti *et al.*, 2021). It's presence in food products is well established (Aris and Paris, 2010). Glyphosate exposure of F0 gestating females and breeding of the F1 generation offspring to the F2 and F3 generations was used to assess the impacts of glyphosate on male reproductive pathology (Kubsad *et al.*, 2019). The transgenerational pathology included increased testis pathology, prostate pathology, increased weaning weight, and obesity, **Table 1**. Other transgenerational pathologies included kidney disease and multiple disease, **Table 1**. Sperm epigenetic biomarkers were also found for the various transgenerational reproductive pathologies (Ben Maamar *et al.*, 2021a).

As can be seen above, epigenetic transgenerational inheritance of male reproductive pathologies can be induced by ancestral exposure to the different environmental toxicants discussed, **Table 1**. The question is raised if different toxicants promote different epigenetic changes that are transmitted transgenerationally. A study in rats addressed this question by comparing the different exposure transgenerational DNA methylation patterns in the sperm of the transgenerational F3 generation. A comparison of the different exposures for vinclozolin, pesticides, DDT, methoxychlor, and plastics were found each to have distinct sperm DNA methylation, so are exposure specific (Beck *et al.*, 2022; Manikkam *et al.*, 2012a). Therefore, specific transgenerational sperm epimutations were observed and indicated subsets of unique epimutations within a larger set of epimutations were sufficient to promote the reproductive disease, a system epigenetic phenomenon (Beck *et al.*, 2022). In addition to the exposure specific epigenetic epimutations (e.g., differential DNA methylation regions, DMRs), disease specific epimutations and DMRs were also identified within the sperm epigenetics that mediate the epigenetic transgenerational inheritance of the phenomenon (Beck *et al.*, 2022; Ben Maamar *et al.*, 2021b). Therefore, the role of environmental epigenetics in the etiology of reproductive disease is being elucidated.

Conclusions

Studies in rodents have shown environmental toxicants can induce epigenetic alterations that are transgenerationally inherited to promote male reproductive pathologies. The epigenetic transgenerational inheritance phenomenon has now been demonstrated in all organisms investigated from plants to humans (Nilsson *et al.*, 2022). Human studies have identified epigenetic biomarkers for disease (Skinner, 2024). Therefore, ancestral exposures to environmental toxicants and other factors such as diet all have the capacity to promote the epigenetic transgenerational inheritance of male reproductive disease, and others. The transgenerational induction of disease susceptibility within the population is likely a major factor in our high pathology rates observed within the population today. The detection of these epigenetic alterations early in life may allow preventative medicine to be used in the future to reduce this male reproductive disease burden.

References

Anway, M.D., Cupp, A.S., Uzumcu, M., Skinner, M.K., 2005. Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 308, 1466–1469.

Anway, M.D., Memon, M.A., Uzumcu, M., Skinner, M.K., 2006. Transgenerational effect of the endocrine disruptor vinclozolin on male spermatogenesis. J. Androl. 27, 868–879.

Anway, M.D., Skinner, M.K., 2006. Epigenetic transgenerational actions of endocrine disruptors. Endocrinology 147, S43–S49.

Aoyama, H., Chapin, R.E., 2014. Reproductive toxicities of methoxychlor based on estrogenic properties of the compound and its estrogenic metabolite, hydroxyphenyltrichloroethane. Vitam. Horm. 94, 193–210.

Aris, A., Paris, K., 2010. Hypothetical link between endometriosis and xenobiotics-associated genetically modified food. Gynecol. Obstet. Fertil. 38, 747–753.

Beck, D., Ben Maamar, M., Skinner, M.K., 2021. Integration of sperm ncRNA-directed DNA methylation and DNA methylation-directed histone retention in epigenetic transgenerational inheritance. Epigenetics Chromatin 14, 6.

Beck, D., Nilsson, E.E., Ben Maamar, M., Skinner, M.K., 2022. Environmental induced transgenerational inheritance impacts systems epigenetics in disease etiology. Sci. Rep. 12, 5452.

Bukowska, B., Wozniak, E., Sicinska, P., Mokra, K., Michalowicz, J., 2022. Glyphosate disturbs various epigenetic processes in vitro and in vivo - A mini review. Sci. Total Environ. 851, 158259.

Van Cauwenbergh, O., Di Serafino, A., Tytgat, J., Soubry, A., 2020. Transgenerational epigenetic effects from male exposure to endocrine-disrupting compounds: A systematic review on research in mammals. Clin. Epigenetics 12, 65.

CDC, 2016. Dichlorodiphenyltrichloroethane (DDT) [Online]. Available: https://www.cdc.gov/biomonitoring/ddt_factsheet.html [Accessed August 31 2017].

Chen, Q., Yan, M., Cao, Z., *et al.,* 2016a. Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. Science 351, 397–400.

Chen, Q., Yan, W., Duan, E., 2016b. Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications. Nat. Rev. Genet. 17, 733–743.

Guerrero-Bosagna, C., Savenkova, M., Haque, M.M., Nilsson, E., Skinner, M.K., 2013. Environmentally induced epigenetic transgenerational inheritance of altered sertoli cell transcriptome and epigenome: Molecular etiology of male infertility. PLoS ONE 8, 1–12 e59922.

Guerrero-Bosagna, C., Settles, M., Lucker, B.J., Skinner, M.K., 2010. Epigenetic transgenerational actions of vinclozolin on promoter regions of the sperm epigenome. Plos One 5, 1–17 e13100.

He, Y., Yin, R., 2024. The reproductive and transgenerational toxicity of microplastics and nanoplastics: A threat to mammalian fertility in both sexes. J. Appl. Toxicol. 44, 66–85.

Holliday, R., Pugh, J.E., 1975. DNA modification mechanisms and gene activity during development. Science 187, 226–232.

Huang, T., Zhang, W., Lin, T., *et al.,* 2022. Maternal exposure to polystyrene nanoplastics during gestation and lactation induces hepatic and testicular toxicity in male mouse offspring. Food Chem. Toxicol. 160, 112803.

King, S.E., Mcbirney, M., Beck, D., *et al.,* 2019. Sperm epimutation biomarkers of obesity and pathologies following DDT induced epigenetic transgenerational inheritance of disease. Environ. Epigenet. 5, 1–15 dvz008.

Klukovich, R., Nilsson, E., Sadler-Riggleman, I., *et al.,* 2019. Environmental toxicant induced epigenetic transgenerational inheritance of prostate pathology and stromal-epithelial cell epigenome and transcriptome alterations: Ancestral origins of prostate disease. Sci. Rep. 9, 1–17.

Kornfeld, J.W., Bruning, J.C., 2014. Regulation of metabolism by long, non-coding RNAs. Front. Genet. 5, 57.

Kubsad, D., Nilsson, E.E., King, S.E., *et al.,* 2019. Assessment of glyphosate induced epigenetic transgenerational inheritance of pathologies and sperm epimutations: Generational toxicology. Sci. Rep. 9, 6372.

Lorz Pm, T.O.W.A.E.F.K., Enke W, J.Ä.C.K.H.R., Bhargava, N., W., H, 2002. Phthalic acid and derivatives. Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH, Weinheim.

Ben Maamar, M., Beck, D., Nilsson, E.E., Kubsad, D., Skinner, M.K., 2021a. Epigenome-wide association study for glyphosate induced transgenerational sperm DNA methylation and histone retention epigenetic biomarkers for disease. Epigenetics 16, 1150–1167.

Ben Maamar, M., Nilsson, E.E., Skinner, M.K., 2021c. Epigenetic transgenerational inheritance, gametogenesis and germline development. Biol. Reprod. 105, 570–592.

Ben Maamar, M., Nilsson, E., Thorson, J.L.M., Beck, D., Skinner, M.K., 2021. Transgenerational disease specific epigenetic sperm biomarkers after ancestral exposure to dioxin. Environ. Res. 192, 110279.

Ben Maamar, M., Sadler-Riggleman, I., Beck, D., *et al.,* 2018. Alterations in sperm DNA methylation, non-coding RNA expression, and histone retention mediate vinclozolin-induced epigenetic transgenerational inheritance of disease. Environ. Epigenet. 4, 1–19 dvy010.

Manikkam, M., Guerrero-Bosagna, C., Tracey, R., Haque, M.M., Skinner, M.K., 2012a. Transgenerational actions of environmental compounds on reproductive disease and identification of epigenetic biomarkers of ancestral exposures. PLoS One 7, 1–12 e31901.

Manikkam, M., Haque, M.M., Guerrero-Bosagna, C., Nilsson, E., Skinner, M.K., 2014. Pesticide methoxychlor promotes the epigenetic transgenerational inheritance of adult onset disease through the female germline. PLoS One 9, 1–19 e102091.

Manikkam, M., Tracey, R., Guerrero-Bosagna, C., Skinner, M., 2012b. Pesticide and insect repellent mixture (Permethrin and DEET) induces epigenetic transgenerational inheritance of disease and sperm epimutations. Reprod. Toxicol. 34, 708–719.

Manikkam, M., Tracey, R., Guerrero-Bosagna, C., Skinner, M., 2013. Plastics derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. PLoS One 8, 1–18 e55387.

Manikkam, M., Tracey, R., Guerrero-Bosagna, C., Skinner, M.K., 2012c. Dioxin (TCDD) induces epigenetic transgenerational inheritance of adult onset disease and sperm epimutations. PLoS One 7, 1–15 e46249.

Milesi, M.M., Lorenz, V., Durando, M., Rossetti, M.F., Varayoud, J., 2021. Glyphosate herbicide: Reproductive outcomes and multigenerational effects. Front. Endocrinol. 12, 672532.

Nilsson, E.E., Ben Maamar, M., Skinner, M.K., 2022. Role of epigenetic transgenerational inheritance in generational toxicology. Environ. Epigenet. 8, 1–9 dvac001.

Nilsson, E., Larsen, G., Manikkam, M., *et al.,* 2012. Environmentally induced epigenetic transgenerational inheritance of ovarian disease. PLoS One 7, 1–18 e36129.

Nilsson, E., Thorson, J.L.M., Ben Maamar, M., Beck, D., Skinner, M.K., 2020. Epigenome-wide association study (EWAS) for potential transgenerational disease epigenetic biomarkers in sperm following ancestral exposure to the pesticide methoxychlor. Environ. Epigenet. 6, 1–25 dvaa020.

Osimitz, T.G., Grothaus, R.H., 1995. The present safety assessment of deet. J. Am. Mosq. Control Assoc. 11, 274–278.

Riggs, A.D., 1975. X inactivation, differentiation, and DNA methylation. Cytogenet Cell Genet. 14, 9–25.

Rossetti, M.F., Canesini, G., Lorenz, V., *et al.,* 2021. Epigenetic changes associated with exposure to glyphosate-based herbicides in mammals. Front. Endocrinol. 12, 671991.

Rothbart, S.B., Strahl, B.D., 2014. Interpreting the language of histone and DNA modifications. Biochim. Biophys. Acta 1839, 627–643.

Sadler-Riggleman, I., Klukovich, R., Nilsson, E., *et al.,* 2019. Epigenetic transgenerational inheritance of testis pathology and sertoli cell epimutations: Generational origins of male infertility. Environ. Epigenet. 5, 1–18 dvz013.

Schuster, A., Skinner, M.K., Yan, W., 2016. Ancestral vinclozolin exposure alters the epigenetic transgenerational inheritance of sperm small noncoding RNAs. Environ. Epigenet. 2, 1–10 dvw001.

Singer, J., Roberts-Ems, J., Riggs, A.D., 1979a. Methylation of mouse liver DNA studied by means of the restriction enzymes msp I and hpa II. Science 203, 1019–1021.

Singer, J., Schnute, W.C., J.R., Shively, J.E., Todd, C.W., Riggs, A.D., 1979b. Sensitive detection of 5-methylcytosine and quantitation of the 5-methylcytosine/cytosine ratio in DNA by gas chromatography–mass spectrometry using multiple specific ion monitoring. Anal. Biochem. 94, 297–301.

Skinner, M., Guerrero-Bosagna, C., Haque, M.M., *et al.,* 2013a. Environmentally induced transgenerational epigenetic reprogramming of primordial germ cells and subsequent Germline. PLoS One 8, 1–15 e66318.

Skinner, M.K., 2011. Environmental epigenetic transgenerational inheritance and somatic epigenetic mitotic stability. Epigenetics 6, 838–842.

Skinner, M.K., 2015. Environmental epigenetics and a unified theory of the molecular aspects of evolution: A neo-lamarckian concept that facilitates neo-darwinian evolution. Genome Biol. Evol. 7, 1296–1302.

Skinner, M.K., 2024. Epigenetic biomarkers for disease susceptibility and preventative medicine. Cell Metab. 36, 263–277.

Skinner, M.K., Manikkam, M., Guerrero-Bosagna, C., 2010. Epigenetic transgenerational actions of environmental factors in disease etiology. Trends Endocrinol. Metab. 21, 214–222.

Skinner, M.K., Manikkam, M., Tracey, R., *et al.,* 2013b. Ancestral dichlorodiphenyltrichloroethane (DDT) exposure promotes epigenetic transgenerational inheritance of obesity. BMC Med. 11, 1–16 228.

Skinner, M.K., Nilsson, E.E., 2021. Role of environmentally induced epigenetic transgenerational inheritance in evolutionary biology: Unified evolution theory. Environ. Epigenet. 7, 1–12 dvab012.

Skinner, M.K., Savenkova, M., Zhang, B., Gore, A.C., Crews, D., 2014. Gene bionetworks involved in epigenetic transgenerational inheritance of altered mate preference: Environmental epigenetics and evolutionary biology. BMC Genomics 15, 337.

Thorson, J.L.M., Beck, D., Ben Maamar, M., Nilsson, E., Skinner, M.K., 2020. Epigenome-wide association study for pesticide (Permethrin and DEET) induced DNA methylation epimutation biomarkers for specific transgenerational disease. Environ. Health 19, 109.

Thorson, J.L.M., Beck, D., Ben Maamar, M., Nilsson, E.E., Skinner, M.K., 2021. Ancestral plastics exposure induces transgenerational disease-specific sperm epigenome-wide association biomarkers. Environ. Epigenet. 7, 1–13 dvaa023.

Tracey, R., Manikkam, M., Guerrero-Bosagna, C., Skinner, M., 2013. Hydrocarbons (jet fuel JP-8) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. Reprod. Toxicol. 36, 104–116.

Wang, X., Martinez, M.A., Dai, M., *et al.,* 2016. Permethrin-induced oxidative stress and toxicity and metabolism. A review. Environ. Res. 149, 86–104.

Wei, J.W., Huang, K., Yang, C., Kang, C.S., 2017. Non-coding RNAs as regulators in epigenetics (Review). Oncol. Rep. 37, 3–9.

WHO, 2010. World Health Organization WHO Ten Chemicals of Major Public Health Concern. http://www.who.int/ipcs/assessment/public_health/chemicals_phc/en/ [Online]. [Accessed 17 May 2015].

Witzmann, F.A., Bobb, A., Briggs, G.B., *et al.,* 2003. Analysis of rat testicular protein expression following 91-day exposure to JP-8 jet fuel vapor. Proteomics 3, 1016–1027.

Wu, T.P., Wang, T., Seetin, M.G., *et al.,* 2016. DNA methylation on N(6)-adenine in mammalian embryonic stem cells. Nature 532, 329–333.

Yaniv, M., 2014. Chromatin remodeling: From transcription to cancer. Cancer Genet. 207, 352–357.