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Authors: Nilsson, Eric E., and Skinner, Michael K.

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Minireview

Environmentally Induced Epigenetic Transgenerational Inheritance of Reproductive Disease[1]

Eric E. Nilsson and Michael K. Skinner[2]

Center for Reproductive Biology, School of Biological Sciences, Washington State University, Pullman, Washington

ABSTRACT

Reproductive disease and fertility issues have dramatically increased in the human population over the last several decades, suggesting environmental impacts. Epigenetics provides a mechanistic link by which an organism can respond to environmental factors. Interestingly, environmentally induced epigenetic alterations in the germ line can promote aberrant gene expression and disease generationally. Environmentally induced epigenetic transgenerational inheritance is defined as germ-line transmission of altered epigenetic information between generations in the absence of continued environmental exposures. This form of nongenetic inheritance has been shown to directly influence fertility and reproductive disease. This review describes the studies in a variety of species that impact reproductive disease and abnormalities. Observations suggest serious attention be paid to the possibility that ancestral exposures to environmental insults promotes transgenerational inheritance of reproductive disease susceptibility. Environmentally induced epigenetic transgenerational inheritance appears to be an important contributing factor to reproductive disease in many organisms, including humans.

developmental biology, environment, epigenetics, genomics

INTRODUCTION

Fertility issues have been increasing in human populations for decades. In men, there have been decreases in sperm count [1–4], increases in testicular cancer [5], and increases in genital abnormalities [6]. Fecundity rates in men and women in both developing and industrial countries have dramatically decreased in recent years [7, 8]. Although economics, social trends, and governmental policies certainly contribute to a decreased birthrate, attention must be paid to the role of environmental toxicants and other exposures in promoting reproductive disease [9–11]. Correlations have been made between environmental exposures and prostate and mammary disease [12, 13], semen quality [1, 14–16], reproductive developmental abnormalities [17–23], polycystic ovarian syndrome [24], and endometriosis [25, 26]. Therefore, the environment has a significant effect on fertility and reproductive disease etiology [27].

The majority of environmental factors and toxicants do not have the ability to promote DNA mutations [28]. Therefore, molecular mechanisms other than alterations in DNA sequence must mediate the ability of environmental factors to promote reproductive disease. Certainly, there are DNA mutations that are associated with specific disease. For example, polymorphisms in the FSH beta promoter result in low sperm counts [29], and fragile X mental retardation gene 1 CGG repeat abnormalities are associated with the oocyte follicle loss of primary ovarian insufficiency [30]. However, genome-wide association studies have generally shown that less than 2% of specific adult-onset diseased populations have a correlated DNA sequence mutation [31, 32]. In addition, the genetic background in human populations is essentially static, while increases in disease disorders and infertility are dramatically increasing [12]. Therefore, environmental exposures must act primarily through epigenetic mechanisms to promote reproductive disease [33, 34].

The term ''epigenetics'' was coined by Dr. Conrad Waddington, University of Edinburgh, in the 1940s to describe gene-environment interactions that could not be explained with classic genetics [35]. Using a more recent mechanistic definition, ''epigenetics'' is defined as molecular factors/processes around the DNA that regulate genome activity independent of DNA sequence and that are mitotically stable [33]. In the 1970s, the first epigenetic molecular mark was identified as being DNA methylation, in which a small chemical (methyl) group is attached to DNA at primarily the cytosine base in animals [36, 37]. In the 1990s, the histone proteins around which DNA is wrapped were also found to be chemically modified to alter gene expression. In the 2000s, noncoding RNA molecules were identified that can act as epigenetic factors [38]. The coiling, looping, and general structure of DNA, termed ''chromatin structure,'' is also an epigenetic factor [39]. Therefore, the currently known epigenetic molecular processes are DNA methylation, histone modifications, functional noncoding RNA, and chromatin structure [27]. All of these processes are a normal part of physiology and cell differentiation and are a part of the cellular machinery that regulates gene expression. Epigenetic processes are often the mechanistic link by which an organism can respond to its environment and change gene expression. Abnormal epigenetic mechanisms can result in alterations in gene expression patterns. These epigenetic abnormalities can lead to aberrant physiology and disease.

[1]Supported by an NIEHS grant to M.K.S.
[2]Correspondence: Michael K. Skinner, Center for Reproductive, Biology, School of Biological Sciences, Washington State University, Pullman, WA 99164-4236. E-mail: skinner@wsu.edu

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EPIGENETIC CHANGES ASSOCIATED WITH REPRODUCTIVE DISEASE

Previous studies have shown that many cases of reproductive disease are accompanied by changes in the epigenome. In males, the incidence of germ cell tumors is correlated with epigenetic disruption [40]. The sperm of infertile men has been shown to have abnormal DNA methylation, histone modifications, and an altered retention of histones in contrast to the usual replacement of histones with protamines [41–43]. In adult rat testes that show an increase in germ cell apoptosis, the isolated Sertoli cells showed changes in the DNA methylation at specific gene promoters as well as accompanying changes in their transcriptome [44]. Prostate tumor growth and invasiveness have been linked to abnormal expression of the polycomb repressor complex that induces changes in chromatin structure that are needed for the formation of heterochromatin [45].

In females, several reproductive diseases have also been associated with epigenetic abnormalities. The occurrence of endometriosis has been linked to the epigenetic deregulation of ''endometriosis susceptibility'' genes [46–48]. Women exposed in utero to diethylstilbesterol develop cervical malformations. These malformations are correlated with DNA methylation changes of *Hox* genes [49, 50]. Epigenetic changes may underlie some of the decrease in oocyte quality that occur as women age [51]. Embryo growth and development can be negatively impacted if the dietary methyl donors and cofactors required for normal DNA and histone methylation processes are deficient [52]. In rat ovaries that show a loss of oocytes, isolated granulosa cells showed changes in the DNA methylation at specific gene promoters as well as accompanying changes in their transcriptome [53]. Taken together, there is strong evidence that epigenetic abnormalities are linked to reproductive disease.

EPIGENETIC TRANSGENERATIONAL INHERITANCE

Environmentally induced epigenetic transgenerational inheritance is defined as the germ-line transmission of altered epigenetic information between generations in the absence of continued environmental exposures [10, 54]. These epigenetic germ-line alterations will subsequently affect gene expression and epigenetic programming patterns in somatic tissues [10, 44] (Fig. 1). The first mammalian example of an environmentally induced epigenetic transgenerational inheritance process was described in 2005 using an early developmental exposure to the endocrine disruptor vinclozolin [55]. Vinclozolin is an agricultural fungicide with antiandrogenic activity widely used in fruit and vegetable crops around the world [56]. Exposure of pregnant rats to vinclozolin produced increased apoptosis in spermatogenic cells, which was observed in each of the four generations after this initial exposure [55, 57, 58] (Fig. 1). The mechanism involved in the transgenerational transmission of these altered phenotypes was an induced alteration in the sperm epigenome that was observed three generations after the developmental exposure to vinclozolin [55, 59, 60].

In considering transgenerational phenomena, it is important to distinguish between direct exposure effects versus germ-line (sperm or egg)-mediated transgenerational events. When a gestating F0 generation female is exposed the F0 generation female, the F1 generation fetus and the germ cell (sperm or egg) that is inside the fetus and that will produce the F2 generation are all directly exposed (Fig. 1). Any effects in the F0, F1, and F2 generations may be due to direct exposure toxicity or to environmentally induced epigenetic changes in the directly exposed cells. Examination of the F3 generation (great grand-offspring) is needed to determine if a transgenerational phenomenon has occurred since the F3 generation has had no direct exposure effects [61]. In contrast, in the event an adult male or nonpregnant female is exposed, the F0 generation adult and the germ cells that will generate the F1 generation are directly exposed such that examination of the F2 generation (grand-offspring) is required to demonstrate a transgenerational phenomenon [61].

In order for a transgenerational effect to occur, it is critical that the germ-line epigenome be altered to allow for transmission to future generations. There are several critical stages of germ cell development where dramatic epigenetic programming occurs [62, 63]. These periods in germ cell development and epigenetic programming represent critical windows of sensitivity to environmental factors [10, 27]. The first is when the stem cells (precursor cells) for the germ cells, called primordial germ cells, develop and migrate during the time of gonadal sex determination at the onset of testis and ovary development. The DNA methylation of those primordial germ cells is predominantly erased, and then subsequently remethylation is initiated during testis and ovary maturation (Fig. 2). The second period is when the sperm and egg come together at fertilization and the DNA contributed by the sperm and egg again are demethylated to create the embryonic stem cells [62, 63] (Fig. 2). This epigenetic programming allows the embryonic cells to develop pluripotency. Interestingly, when exposures to toxicants or abnormal nutrition occur during gonadal sex determination, the epigenetic programming or DNA methylation of the germ cell can become reprogrammed and transmit altered epigenetic information transgenerationally to subsequent generations [10, 55].

TRANSGENERATIONAL EPIGENETIC REPRODUCTIVE DISEASE

There are now several studies in mammals reporting the occurrence of environmentally induced transgenerational epigenetic inheritance of reproductive disease. Table 1 lists environmental exposures that can result in inheritance of epigenetic transgenerational abnormalities and highlights those that promote reproductive disease states. As shown, the majority of studies have associated epigenetic changes reported. In male rat testis, abnormalities observed included decreased sperm counts and impaired motility, which have been shown to be transgenerationally inherited after ancestral exposure to vinclozolin [55, 58] and DDT [64, 65]. In mice, similar transgenerational decreases in sperm production were seen after ancestral exposure to phthalates, which are components of plastics, cosmetics, and other products [66]. An increase in germ cell apoptosis was seen in rat testes transgenerationally after ancestral exposure to vinclozolin [55], DDT [64], and a jet fuel/hydrocarbon mixture [67]. In mice, transgenerational inheritance of testis germ cell apoptosis has also been reported after ancestral exposure to vinclozolin [59, 65]. Other testis abnormalities that have shown transgenerational epigenetic inheritance include an increased incidence of histologically detected seminiferous tubule atrophy, tubule vacuoles and germ cell agenesis, which occured after ancestral exposure of rats to vinclozolin [58], a mixture of the plasticizers bisphenol A (BPA) and phthalates [68], and the combination of the insecticide permethrin and the insect repellent DEET [69]. In mice, similar histologic seminiferous tubule defects were seen after ancestral exposure to phthalates [66] and the industrial environmental contaminant benzo[*a*]-pyrene [70]. In rat testes that exhibit a transgenerational increase in germ cell apoptosis after ancestral vinclozolin exposure, isolated Sertoli cells show correlated alterations in

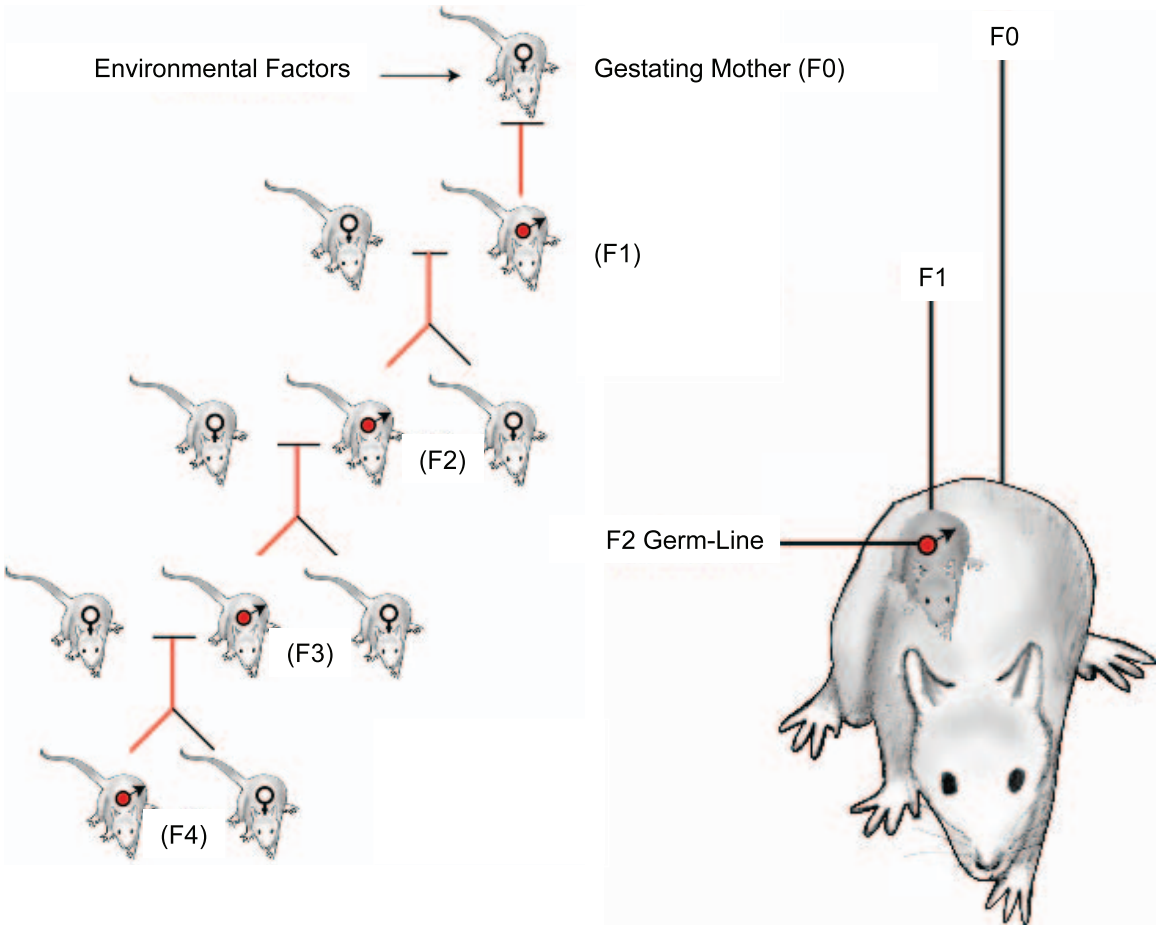

FIG. 1. Environmentally induced epigenetic transgenerational inheritance through male germ line. Exposure of the F0 generation gestating female, F1 generation fetus, and germ line within the F1 generation fetus that will generate the F2 generation. Therefore, the F3 generation is the first transgenerational generation not directly exposed. Figure modified from Skinner [61].

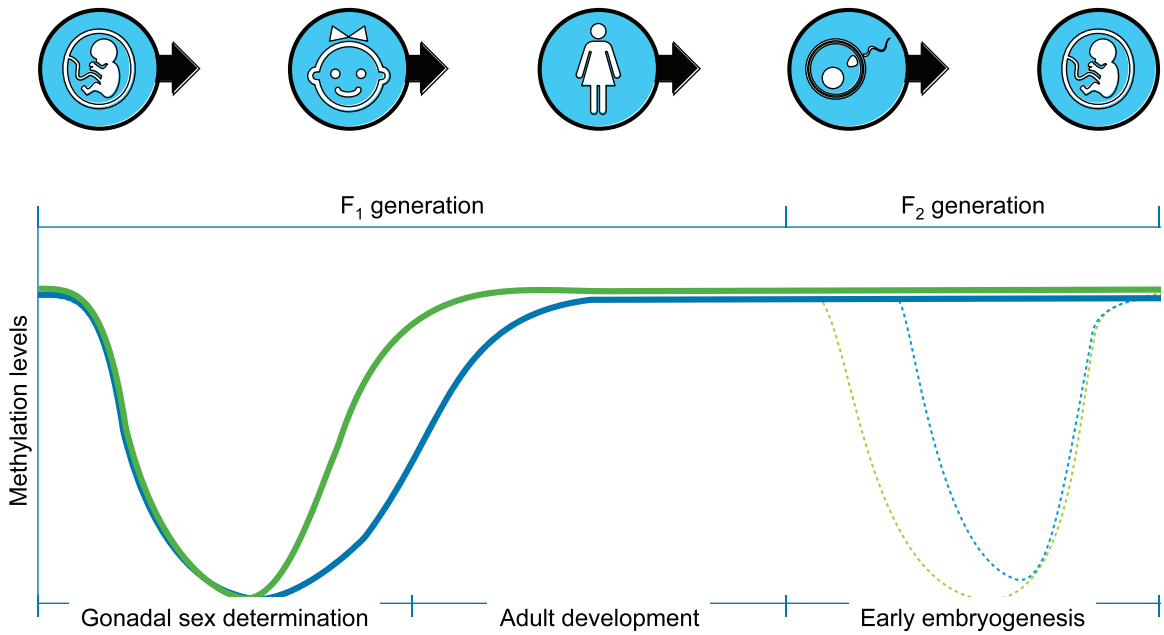

FIG. 2. Epigenetic programming of DNA methylation levels during development during gonadal sex determination, fertilization, and embryonic development. The green line is the male and the blue line the female developmental pattern. Figure modified from Jirtle and Skinner [27].

TABLE 1. Environmental exposures that induce transgenerational reproductive disease phenotypes.

Toxicants	Reproductive disease	References
Vinclozolin	Decreased sperm count, testis apoptosis, testis abnormalities,[a] prostate abnormalities, oocyte loss, ovarian cysts, altered mate selection. Epigenetic changes observed.	[51, 55–57, 63, 69, 78]
Methoxychlor	Ovarian cysts. Epigenetic changes observed.	[55, 75, 80]
TCDD/dioxin	Puberty onset, oocyte loss, ovarian cysts, fertility defect.[b] Epigenetic changes observed.	[72, 77, 78]
Plastics mixture (bisphenol-A, phthalate-DEHP, and DBP)	Testis abnormalities, puberty onset, oocyte loss, ovarian cysts. Epigenetic changes observed.	[67, 68]
Jet fuel (JP8)	Testis apoptosis, oocyte loss. Epigenetic changes observed.	[65, 74]
Permethrin and DEET	Testis abnormalities, puberty onset, oocyte loss, ovarian cysts. Epigenetic changes observed.	[69]
DDT	Decreased sperm count, testis apoptosis, ovarian cysts. Epigenetic changes observed.	[63, 64]
Bisphenol A	Decreased sperm count, fertility defect	[79, 89, 90]
Phthalates	Decreased sperm count, testis abnormalities, puberty onset, fertility defect	[66]
Tributyltin		[91]
Benzo[a]pyrene	Testis abnormalities	[70]
Other types exposures		
Folate (nutrition)		[92]
High-fat diet (nutrition)		[93, 94]
Caloric Restriction (nutrition)		[95–98]
Temperature and drought (plant flowering and health)	Abnormal flowering, fertility defect. Epigenetic changes observed.	[99–102]
Stress (behavioral)		[103, 104]
Smoking (health)		[105, 106]
Alcohol (health)		[107]

[a] Includes seminiferous tubule atrophy, tubule vacuoles, and germ cell agenesis.
[b] Fertility defect indicates reduced numbers of offspring.

both DNA methylation (epigenome) and RNA expression (transcriptome) [44].

Prostate gland abnormalities, including prostate epithelial hyperplasia and epithelial atrophy, have been transmitted transgenerationally in rats and mice after ancestral exposure to vinclozolin. This was accompanied by transgenerational changes in RNA expression in prostate epithelial cells [59, 71].

Ancestral exposure to environmental toxicants can induce transgenerational changes to the timing of the onset of puberty. In rats, exposure of pregnant F0 generation females to a mixture of BPA and phthalates [68], a combination of permethrin and DEET [69], or the industrial contaminant dioxin [72] induced changes in the time of puberty onset in the unexposed F3 generation. Similar transgenerational changes in the onset of puberty were seen in mice after ancestral exposure to phthalates [66].

A female reproductive disease that has shown transgenerational epigenetic inheritance is a decreased follicle pool associated with decreases in total oocytes per ovary [67]. Follicle loss such as this is a major component of the human disease condition primary ovarian insufficiency, which results in premature menopause [73]. Rats have shown transgenerational epigenetic inheritance of decreases in the follicle pool after ancestral exposures to jet fuel hydrocarbons [74], a mixture of BPA and phthalates [68], dioxin [72], permethrin/DEET [69], and vinclozolin [53]. Similarly, an increase in the incidence of ovarian cysts was transmitted to the F3 generation after ancestral exposure to the pesticide methoxychlor [75], DDT [64], a mixture of plasticizers [68], dioxin [72], permethrin/DEET [69], and vinclozolin [53]. In women, polycystic ovarian syndrome is a common problem resulting in infertility and is increasing in frequency [76]. In rat ovaries that exhibit a transgenerational decrease in their follicle pool and an increase in ovarian cysts after ancestral vinclozolin exposure, isolated granulosa cells show correlated alterations in both DNA methylation and RNA expression [53].

Several studies have documented a transgenerational decrease in fertility as measured by the number of offspring produced following ancestral exposure to environmental toxicants. Pregnant F0 generation mice exposed to dioxin had female progeny of the F1, F2, F3, and F4 generations with an increased incidence of preterm birth and decreased fertility [77]. Dioxin also was shown to decrease fertility transgenerationally in zebrafish [78]. Exposure of pregnant mice to phthalates also induced the transgenerational inheritance of reduced fertility [66]. Similarly, pregnant rats exposed perinatally to BPA gave birth to offspring with significantly impaired spermatogenesis and fertility, and there was transgenerational inheritance of this fertility defect in male progeny [79].

Ancestral exposure to toxicants can induce transgenerational inheritance of an altered mate preference that is associated with sexual selection. The exposure of F0 generation pregnant rats to vinclozolin resulted in the transgenerational F3 generation females showing an altered mate preference [80]. This demonstrates that transgenerational inheritance of epigenetically mediated changes could affect sexual selection and evolution [81].

Epigenetic transgenerational inheritance of disease susceptibility can be induced by ancestral exposure to several different environmental toxicants (Table 1). The question is raised whether different toxicants promote different epigenetic changes that are passed transgenerationally. A study in rats addressed this question by comparing the DNA methylation patterns in the sperm of the F3 generation progeny of F0 generation gestating females that had been exposed to either jet fuel hydrocarbons, dioxin, a plastics mixture, or a pesticide mixture [67]. Interestingly, the sites of changes in DNA methylation, known as differentially methylated regions (DMR), formed a unique pattern with each different ancestral toxicant exposure (Fig. 3). No transgenerational DMR was common to all the exposures. This suggests that DNA

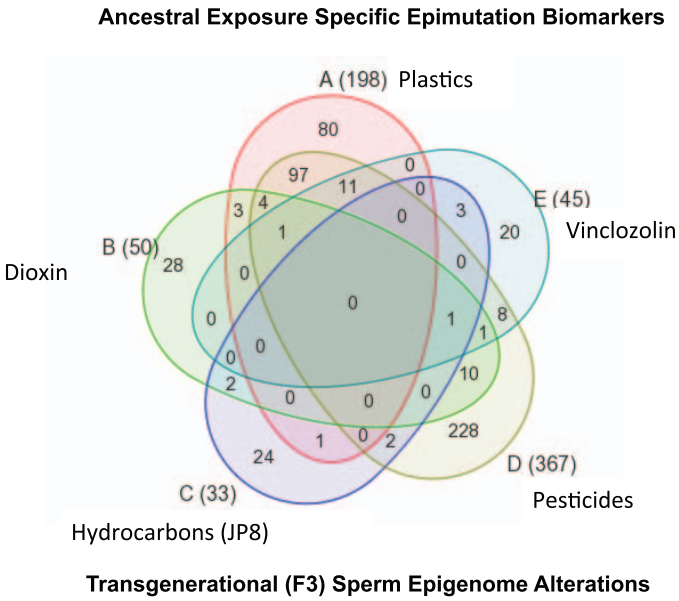

FIG. 3. Ancestral exposure specific epimutation biomarkers. Transgenerational F3 generation sperm differential DNA methylation regions (epimutations) with the total listed next to exposure in brackets and Venn diagram showing overlap between the exposure epimutations. Figure modified from Manikkam et al. [67].

methylation patterns could be used as biomarkers to detect ancestral exposure to environmental toxicants. In the future, individuals so tested might then be able to predict susceptibility to later-life correlated disease. Further research is needed to confirm the accuracy of such biomarkers and determine what effect variables such as toxicant dose have on inherited epigenetic alterations.

BIOLOGICAL IMPACTS AND MECHANISMS

Studies have demonstrated that epigenetic transgenerational inheritance of an increased susceptibility for reproductive disorders can occur after ancestral exposure to an environmental insult. Interestingly, transgenerational inheritance of many of the reproductive disorders described in this review may be induced by exposure to any one of several different environmental toxicants or factors (Table 1). For example, transgenerational inheritance of ovarian follicle loss in rats can be induced by ancestral exposure to jet fuel hydrocarbons, BPA, phthalates, dioxin, permethrin, DEET, and vinclozolin [53, 67]. This phenomenon of several toxicants inducing the same transgenerational reproductive disease occurs even though the epigenetic changes induced are different for each toxicant. Therefore, one would expect that different genes would have altered regulation for each toxicant. This phenomenon may be due to the systems biology of the reproductive tissue or organ system that is transgenerationally affected. If expression of enough of the genes in a vital signaling network is disrupted, then a particular disease manifests, regardless of the specific genes affected.

From Table 1, it may be noted that many of the environmental toxicants that can promote epigenetic transgenerational disease could be considered endocrine-disrupting compounds. Endocrine disruptors act to interfere with normal hormone signaling pathways and can have effects at very low doses due to nonmonotonic dose responses [82, 83]. However, it was found that the epigenetic transgenerational effect of vinclozolin in rats was not due to its antiandrogenic actions [65]. Therefore, toxicants may not need to be endocrine disruptors in order to induce epigenetic transgenerational changes.

It is important to remember that epigenetic mechanisms are a crucial part of normal biology and are some of the primary drivers of cell differentiation to produce all the different cell types in an organism [84, 85]. In addition, the epigenome is a normal mechanism by which organisms respond to changes in the environment by changing gene expression. Why, then, can environmental insults result in transgenerational inheritance of increased disease susceptibility, which is a maladaptive response? One possibility may be explained in terms of an environmental mismatch or the predictive adaptive response hypothesis [86–88]. In this hypothesis, an environmental stressor like famine may epigenetically promote an adaptive (thrifty) phenotype in progeny. If the current environment of those progeny has more-than-adequate nutrients, diseases like diabetes and obesity are promoted. Another possibility is that an environmental insult, such as exposure to a toxicant, may interfere with the normal molecular epigenetic machinery and result in stochastic and/or directed epigenetic changes that could be considered epimutations. If these epimutations occur in germ cells, then that can lead to transgenerational inheritance of a wider range of phenotypes in the progeny. Some of those phenotypes may be poorly adapted and develop disease, including reproductive disease. This would explain an increased disease susceptibility in organisms whose ancestors were exposed to environmental insults. However, the increased phenotypic variation may also result in some individuals who are better adapted to an altered environment, facilitating natural selection [81]. These two possibilities are not exclusive of each other, and future investigations will hopefully clarify further how environmental exposures relate to epigenetic transgenerational inheritance of reproductive disease.

CONCLUSIONS

Epigenetic and genetic processes always act in concert to regulate gene expression and physiology of an organism. Epigenetically mediated transgenerational inheritance is a normal physiological process. However, exposure to environmental insults, such as toxicants during critical developmental windows, may result in germ-line inheritance of epimutations and an increased susceptibility to reproductive disease in subsequent generations. In this review of studies in various experimental model species, the reports of epigenetic transgenerational inheritance of reproductive disease and abnormalities are described. These results suggest that serious attention be paid to the possibility that ancestral exposures to environmental insults has led to transgenerational inheritance of increased susceptibility to reproductive problems. This will be an important contributing factor to reproductive disease in most species, including humans. The use of epigenetic biomarkers may allow a diagnostic to be developed to determine the toxicant exposures that occurred in your ancestors, so actions can be taken to help mitigate possible disease onset. Observations suggest that much of the reproductive disease and infertility today may in part be due to ancestral environmental exposures through epigenetic transgenerational inheritance mechanisms.

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