

Invited Review

The epigenetic impacts of endocrine disruptors on female reproduction across generations[†]

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[†]**Grant Support:** This work was supported by the Billie A. Field Fellowship in Reproductive Biology (SR), NIH P01 ES022848 (JAF), EPA RD83 543401 (JAF), NIH F31 ES030467 (SR), and NIH T32 ES007326 (SR).

Edited by Dr. Lane K. Christenson, PhD, University of Kansas Medical Center

Received 8 November 2018; Revised 18 April 2019; Accepted 9 May 2019

Abstract

Humans and animals are repeatedly exposed to endocrine disruptors, many of which are ubiquitous in the environment. Endocrine disruptors interfere with hormone action; thus, causing non-monotonic dose responses that are atypical of standard toxicant exposures. The female reproductive system is particularly susceptible to the effects of endocrine disruptors. Likewise, exposures to endocrine disruptors during developmental periods are particularly concerning because programming during development can be adversely impacted by hormone level changes. Subsequently, developing reproductive tissues can be predisposed to diseases in adulthood and these diseases can be passed down to future generations. The mechanisms of action by which endocrine disruptors cause disease transmission to future generations are thought to include epigenetic modifications. This review highlights the effects of endocrine disruptors on the female reproductive system, with an emphasis on the multi- and transgenerational epigenetic effects of these exposures.

Summary Sentence

Endocrine disruptors alter reproductive tissues and functions across generations via epigenetic mechanisms.

Key words: developmental origins of health and disease, environmental contaminants and toxicants, endocrine disruptors, epigenetics, DNA methylation, female reproductive tract, histone modifications, chromatin.

Endocrine disruptors

Synthetic chemicals have become a part of people's everyday lives and some of these chemicals have been identified as endocrine disruptors. Endocrine disruptors are exogenous chemicals, mixtures of chemicals, or non-chemical exogenous factors that interfere with the body's normal endocrine system, leading to adverse effects on hormonally controlled functions [1]. Endocrine disruptors are heterogeneous and vary from synthetic to natural chemicals. Specifically, synthetic chemicals such as polychlorinated biphenyls, plasticizers, pesticides, fungicides, and pharmaceutical agents are known endocrine disruptors [1]. Natural chemicals such as phytoestrogens found in

food products are also known endocrine disruptors. These chemicals serve various purposes and are ubiquitous in the environment [1]. Endocrine disruptors interfere with hormone actions by mimicking hormones, promoting inappropriate responses at improper times, or by blocking hormone action, leading to alterations in the hormonal and homeostatic systems and interfering with the ability of the body to communicate with and respond to the environment [1]. Endocrine disruptors tend to have a low binding affinity for hormone receptors and their ability to activate or block hormone receptors may vary. Although it is often difficult to define adverse effects, some researchers consider any biological response to an endocrine disruptor to be an adverse event [2].

Endocrine disruptors are found in food, consumer products, water, soil, and in wildlife and people who are exposed through ingestion, inhalation, dermal contact, or injection [1]. Examples of endocrine disruptors vary from chemical to non-chemical exogenous factors [1, 3]. Chemical endocrine disruptors can be categorized into three major groups: pesticides, chemicals in consumer products, and food contact materials [4]. Examples of pesticides that induce endocrine disruptive activities include glyphosate, dichlorodiphenyltrichloroethane (DDT), atrazine, chlorpyrifos, and methoxychlor [4–6]. Endocrine disruptors found in consumer products include, but are not limited to, brominated flame-retardants, phthalates, parabens, heavy metals, polychlorinated biphenyls, nonylphenols, diethylstilbestrol (DES), and perfluorochemicals [4, 7–9]. Additional endocrine disruptors described as food contact materials are bisphenol A (BPA), phthalates, and phenols. [4, 8].

Numerous endocrine disruptors exist, but this review will focus on eight well-documented endocrine disruptors and their epigenetic effects on female reproduction. The chosen chemicals range from chemicals in consumer products, food contact materials, and pesticides and collectively, are ubiquitously found in the environment. Therefore, it is critical to thoroughly investigate and analyze these chemicals across generations on female reproduction. Specifically, BPA is a well described endocrine disruptor [10]. BPA is a synthetic chemical used mostly in polycarbonate plastics, epoxy resin liners in aluminum cans, and thermal receipts. It can act through various sex steroid hormone receptors, including estrogen receptors (ESRs) 1 and 2, androgen receptors, and thyroid hormone receptors [11]. Phthalates are a class of chemicals that serve as plasticizers and act as endocrine disruptors [12, 13]. DES is an endocrine disruptor that was used as an anti-abortion drug until the 1970s, but it is no longer used due to its reproductive toxicity [12]. DDT and its metabolite dichlorodiphenyldichloroethylene (DDE) are organochlorine insecticides and are well known endocrine disruptors [12, 14, 15]. Methoxychlor (MXC) is another organochlorine pesticide and endocrine disruptor that replaced DDT, but methoxychlor is now banned in many countries due to its toxicity [15]. Vinclozolin is a dicarboximide fungicide used in agriculture, but more specifically in the viticulture industry, and it exhibits endocrine disrupting effects [16]. Further, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent polychlorinated dibenzodioxin and endocrine disruptor. It is formed as a by-product of organic combustion and is a major component of the Agent Orange that was used during the Vietnam War. It also is a major environmental contaminant from an industrial explosion in Seveso, Italy [12, 17].

Endocrine disruptors cause non-monotonic dose responses such as sigmoid, U-shaped, or inverted-U-shaped curves [1, 18]. For example, endocrine disruptors that mimic estrogen stimulate MCF-7 human breast cancer cell proliferation at low doses, but saturate the cell growth response and do not increase proliferation at high doses [19]. Although the mechanisms behind such non-monotonic effects are not fully understood, they may be due to receptor type and abundance in specific cells or tissues [20], receptor down-regulation and desensitization [21, 22], and endocrine feedback loops [23, 24].

Endocrine disruptors also have been shown to act at low levels and in the range of normal human exposure [1]. This is not surprising because endocrine disruptors mimic endogenous hormones, which act at low concentrations. Therefore, studies that utilize environmentally relevant and low doses are important for understanding the effects of endocrine disruptors in the body.

Recently, several studies have linked exposure to endocrine disruptors to adverse reproductive outcomes. For example, the incidence and prevalence of diseases involving reproductive tissues such as breast cancer, prostate cancer, and polycystic ovarian syndrome have increased over time [25–27]. These diseases are influenced by both genetic and environmental factors, but dramatic increases in incidence and prevalence suggest that these diseases are largely due to environmental factors. An analysis of about 44,800 pairs of twins from Sweden, Denmark, and Finland shows that the environment, and not genetics, substantially influenced the rates of sporadic prostate and breast cancers [26]. Another study demonstrates that exposure to BPA plays a major role in polycystic ovarian syndrome pathogenesis [25].

The reproductive system is especially vulnerable to endocrine disruptors during development. The developmental origins of health and disease (DOHaD) is a paradigm in which environmental exposures during development can lead to health and disease risk later in childhood and adult life [28]. The concept is that environmental stressors including malnutrition and exposure to environmental endocrine disruptors during critical periods of development cause subtle changes in gene expression that lead to permanent alterations in an organ, tissue, or structure. The alteration will then lead to a health and disease risk later in life [28–30]. In addition, DOHaD disease risk can be transmitted across generations [28].

Endocrine disruptors act through multiple pathways to influence developmental programming. During early development, the fetus is protected from exogenous estrogens by a plasma protein, α -fetoprotein, which binds estrogens and protects the fetus. However, some endocrine disruptors bypass α -fetoprotein due to weak binding affinity, subsequently rendering the fetus vulnerable to toxicity [31]. Further, other hormone-binding proteins circulate through the blood and endocrine disruptors may bind to these proteins, disrupting the balance between hormone-binding proteins and endogenous hormones [32]. Further, endogenous hormones may be less bioavailable, whereas the endocrine disruptors are physiologically available, causing inappropriate hormone signaling [32, 33]. The ability of endocrine disruptors to interfere with hormone levels during development is of concern because cell differentiation and tissue development can be adversely impacted by hormone level changes. Subsequently, these tissues can be predisposed to diseases in adulthood and disease can be passed down to future generations [34].

Mechanisms of multigenerational and transgenerational epigenetic inheritance

The ability of endocrine disruptors to influence developmental programming and cause disease and infertility is a major concern, but even more troubling is that some of the effects of endocrine disruptors may be multigenerational or transgenerational in nature. To obtain a multigenerational effect, the phenotype or effect must occur in generations that were directly exposed to the endocrine disruptor during development. However, to observe a transgenerational effect, the phenotype or the effect must be inherited by the generation that was not directly exposed to the endocrine disruptor during development [35].

Multiple exposure paradigms produce multigenerational and transgenerational effects (Figure 1). The first paradigm is by adult life exposure. The F0 generation is exposed to an endocrine disruptor during adult life. During this window, the F1 generation

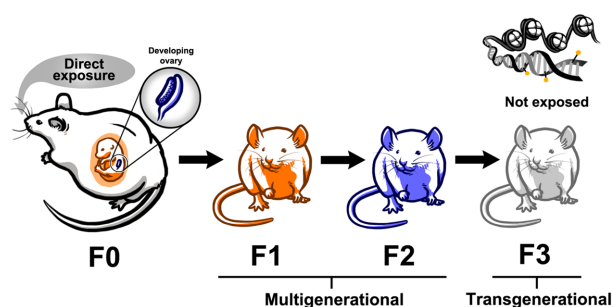


Figure 1. Exposure to endocrine disruptors during prenatal development causes multigenerational effects in the F1 and F2 generations and transgenerational effects in the F3 generation. The F1 and F2 generations are directly exposed to the endocrine disruptor as a fetus and germ cell, respectively. The F3 generation is not directly exposed and the mechanisms governing the effects in the F3 generation are thought to be epigenetic in nature.

experiences preconception exposure as the germ-line. Once the F0 generation produces the F1 generation, any effects observed in the F1 generation are due to multigenerational effects of endocrine disruptor exposure. To observe a transgenerational effect from adult exposure, the subsequent generation, the F2 generation, must be produced. This is the first generation that is not directly exposed to the endocrine disruptor and any effects observed in this generation are considered transgenerational [35–37].

The second exposure paradigm is by prenatal exposure (Figure 1). The F0 generation is exposed to an endocrine disruptor during pregnancy. During this exposure window, the F1 generation is exposed as a developing fetus and the F2 generation is exposed as the developing germ cells inside the fetus. Both the F1 and F2 generations are directly exposed and effects observed in these generations are considered multigenerational effects. The subsequent generation, the F3 generation, is the first generation that is not directly exposed to the chemical and any effects observed are considered transgenerational effects [35–37]. The transgenerational phenomenon does not involve direct exposure and usually involves epigenetic changes induced in the germline [34, 38–40].

Epigenetics are mitotically and meiotically heritable changes in gene function without changing DNA sequences [41, 42]. Broadly, these heritable changes in the epigenome define and control cell and tissue development by controlling gene expression [43]. Multiple molecular mechanisms alter the epigenome, including changes DNA methylation, chromatin modification, and some noncoding RNAs (ncRNAs) (Figure 2). Epigenetic modifications must be transmitted through the germline to the unexposed generation to cause a transgenerational phenomenon [36].

DNA methylation is a commonly studied epigenetic mechanism [44]. Specifically, methylation of DNA is a highly dynamic modification that occurs on the cytosine residue in “CpG” dinucleotides [45]. Cytosines in 5' promoter region that are methylated hinder the transcription of the gene, thus causing gene silencing. This is because DNA methylation is associated with providing a physical barrier, which impedes transcription factor binding, resulting in downregulation of gene expression [46, 47].

In addition to epigenetic modifications occurring by DNA methylation, they can occur due to chromatin modifications. Chromatin modifications are epigenetic modifications that directly regulate the packaging of DNA. Over 3 billion base pairs are contained in chromosomes and need to fit inside the nucleus. Chromatin serves to compact the DNA while allowing transcription factor access to rel-

evant DNA sequences. Chromatin is made of DNA, histone proteins, and nucleosomes. Histones are critical for condensing DNA and their functions are primarily controlled by modifying the N-terminus, also known as histone tail domains. These histone tails are modifiable by non-histone proteins and these modifications reflect DNA compaction. The type of modification on the specific histone and the position of the modification influences gene expression. Histone tail modifications include acetylation, methylation, proline isomerization, SUMOylation, ubiquitination, phosphorylation, ADP ribosylation, and deamination [48]. Post-translational modifications interact with the histone tails to modify the transcriptional regulatory readout. Post-translational modifications may occur on any of these histones [48].

Another type of epigenetic modification involves ncRNA. Non-coding RNAs are forms of epigenetic modifications that serve house-keeping and regulatory functions and are involved in function. Long noncoding RNAs (lncRNAs) are longer than 200 nucleotides in length, do not encode protein, and mediate gene expression. The mechanisms by which lncRNAs exert their epigenetic effects are diverse. Long ncRNA have the ability to interact with genomic loci and chromatin by forming stable domains for protein binding and chromatin localization. Therefore, the lncRNA interactions allow sequence specific localization of chromatin-modifying complexes and the lncRNAs direct the chromatin-modifying complexes to target genes, thus regulating transcriptional activity [49, 50]. Further, lncRNAs can directly interact with DNMT1 and affect global methylation patterns [51]. Finally, lncRNAs have been shown to interact with other ncRNAs such as miRNA. Specifically, lncRNAs can bind and sequester miRNAs and prevent them from binding to their target mRNAs [52, 53]. Further detailed information about epigenetic mechanisms and modifications is described in several extensive epigenetic reviews [44, 46, 54–59].

Epigenetic effects of endocrine disruptors on female reproduction

Multiple organs are required to facilitate healthy reproduction and studies show that endocrine disrupting chemicals can interfere with the function of these organs. Organs within the hypothalamic-pituitary-ovary axis consist of the hypothalamus, anterior pituitary, and the ovary. A summary of studies demonstrating the multigenerational and transgenerational impacts of endocrine disrupting chemicals on each of these organs are described below.

Epigenetic effects of endocrine disruptors on the hypothalamus

Studies indicate that endocrine disrupting chemicals can target the hypothalamus in the brain, leading to epigenetic changes and transgenerational effects. For example, BPA has been shown to cause transgenerational inheritance in the hypothalamus [60]. Prenatal BPA (5 mg/kg) exposure disrupts the number of ER α -cells in brain regions (bed nucleus of the stria terminalis and anteroventral periventricular nucleus) important for reproductive function in female mice in a transgenerational manner [60]. However, the epigenetic mechanism governing the change in ER α cell numbers was not explored in the studies. Further, perinatal BPA exposure (5 mg/kg) increases the expression of *Meg3*, a maternally expressed lncRNA, in the female hypothalamus of the F3 generation [61]. The expression of *Meg3* is important because it is correlated with the central control of

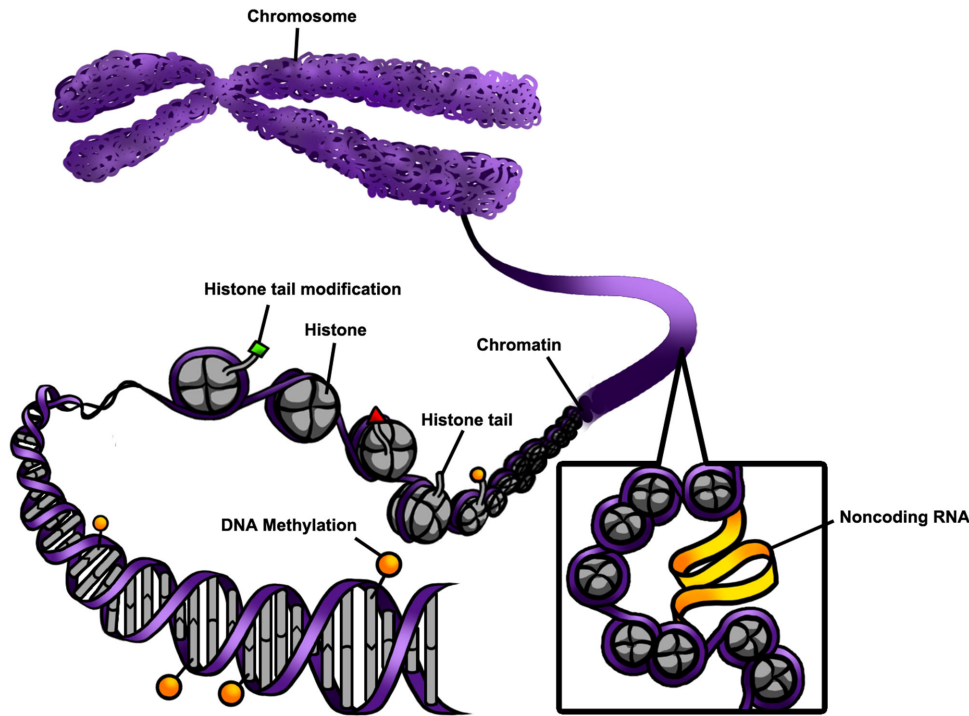


Figure 2. An overview of epigenetic mechanisms including chromatin modifications, DNA methylation, and ncRNA interactions. Chromatin is made of DNA, histone proteins, and nucleosomes and regulates gene expression by controlling the access of transcription factors to DNA. DNA methylation creates a physical barrier that generally impedes transcription factor binding. Noncoding RNA interacts with chromatin and modifies domains.

precocious puberty [62] and increased levels of BPA are associated with precocious puberty in women [63] and in laboratory animals [64]. The increase in *Meg3* is significant because it is an epigenetic modifier and mRNA expression is increased in the generation that was ancestrally exposed to BPA. These findings are significant because the dose of BPA used (5 mg/kg) is estimated to be what is found in human maternal blood (0.3–18.9 ng/mL maternal plasma) and is therefore environmentally relevant [60, 65, 66].

Epigenetic effects of endocrine disruptors on the ovary

Endocrine disruptors can affect several processes in ovary, including the formation of a healthy primordial follicle pool, maintenance of a constant stream of growing follicles, and normal steroidogenic capacity, all of which are required for normal female fertility [13, 67–70]. Any chemical that interferes with these processes can cause severe reproductive outcomes. Specifically, chemicals that target the formation of the primordial follicle pool cause infertility because they deplete the finite follicle reserve used for the growth of ovulatory follicles [67, 68]. Additionally, an increased loss of primordial follicles leads to an early onset of reproductive senescence [69]. This is of concern because early onset of reproductive senescence is associated with increased risk of chronic diseases [69, 71–74]. Chemicals that specifically target primary, preantral, and antral follicles may lead to temporary infertility or permanent infertility. Temporary infertility may occur when the toxicant only targets the mature population of follicles, but not immature follicles. Thus, when the toxicant is removed, the immature follicle types can grow and replenish the mature population of follicles, restoring

fertility. Permanent infertility occurs when the toxicant is not removed and continuously targets the growth and function of ovarian follicles [70]. Permanent infertility is more likely to occur in humans because chemical exposure occurs on a daily basis and it is difficult to remove chemical exposure. Chemicals that target the production of sex steroid hormones from the ovary may lead to infertility and other non-reproductive disorders [70, 71, 75–77].

Exposure to DES has been associated with multigenerational effects on the ovaries in women. In particular, one case study describes small cell carcinoma of the ovary in a 15 year old girl whose maternal grandmother had taken DES during her pregnancy [78]. This study provides one example that prenatal DES exposure is associated with a multigenerational increase in ovarian cancer in the F2 generation in humans. Similarly, another study has shown that prenatal exposure to DES is associated with ovarian cancer in the F2 generation [79]. Unfortunately, the epigenetic mechanisms for these ovarian effects have not been fully investigated across generations. In contrast, some studies have shown that DES exposure does not lead to female genital tract anomalies. In particular, 28 daughters that were F2 generation descendants were evaluated for lower genital tract abnormalities. None of the daughters were found to have abnormalities usually associated with DES exposure [80]. Additionally, a retrospective cohort study of 2268 women exposed to DES in utero shows that their daughters, the F2 generation, did not experience a significant increase in female genital tract anomalies [81]. It is likely that because half of the F2 females were less than 18 years old, the females may not yet show signs of anomalies. Therefore, further evaluation is necessary to understand the effects of DES on female genital tract anomalies in women.

MXC is a banned insecticide once used as a replacement for DDT. It is an endocrine disruptor shown to directly affect ovarian

functions. Studies have shown the exposure to MXC causes various ovarian-related diseases in both multigenerational and transgenerational manners. MXC exposure (200 mg/kg BW/day) causes ovarian disease in the F1 generation of rats and ancestral MXC (200 mg/kg BW/day) exposure increases polycystic ovarian-like syndrome in the F3 generation of rats [82]. Epigenetic analyses show that MXC (100 mg/kg) hypermethylates CpGs in the ER β promoter of the ovary. Further, MXC (100 mg/kg) hypermethylates multiple loci critical for ovarian signaling pathways and concurrently decreases gene expression [83, 84]. MXC (20 μ g and 100 mg/kg) exposure also increases expression of *Dnmt3b* in the ovaries, suggesting that *Dnmt3b* plays a critical role in DNA hypermethylation [83]. The adult general population exposure to MXC ranges between 0.1–0.3 ng/kg/day based on the Food and Drug Administration's Total Diet Study for the period 1986–1991 [85]. Therefore, it is likely that the doses used in previous studies are too high to be relevant to human exposure. Thus, future studies are needed that assess the effects of environmentally relevant levels of methoxychlor on the ovary across generations. Although both multigenerational and transgenerational effects on ovarian functions are observed in experimental animals, it is not yet determined if the epigenetic changes observed in some experiments are specifically linked to an ovarian phenotype. This indicates a need for future studies on epigenetic mechanisms underlying MXC-induced toxicity.

Phthalates are a class of chemicals commonly used as plasticizers, but are also known endocrine disrupting chemicals. Previous studies show that prenatal exposure to a mixture of phthalates (21% di (2-ethylhexyl) phthalate (DEHP), 35% diethyl phthalate (DEP), 15% dibutyl phthalate (DBP), 8% diisobutyl phthalate (DiBP), 5% benzylbutyl phthalate (BzBP), and 15% diisononyl phthalate (DiNP)) that mimics human exposure causes multigenerational effects on mouse ovaries [86, 87]. Specifically, prenatal phthalate mixture exposure (20 μ g/kg/day, 200 μ g/kg/day, 200 mg/kg/day, and 500 mg/kg/day) induces cystic ovaries in the F1 and F2 generations [86, 87]. The phthalate mixture was designed from levels of phthalate metabolites measured in urine samples from pregnant women in Illinois [87, 88]. Additionally, the lowest dose used in the phthalate mixture studies (20 μ g/kg/day) mimics human exposure levels and is within the high end of phthalate detection levels in humans [87]. Another mixture study shows exposure to mixtures of plastic derivatives (BPA 50 mg/kg BW/day, DEHP 750 mg/kg BW/day and DBP 66 mg/kg/BW/day and BPA 25 mg/kg BW/day, DEHP 375 mg/kg BW/day, DBP 33 mg/kg BW/day) causes polycystic ovaries in both the F1 and F3 generations [89]. Although the doses in these studies are high in relation to human exposures, the study was designed to examine pharmacological actions of the chemicals on epigenetic transgenerational inheritance [89].

Exposure to a single phthalate, DEHP, also causes adverse transgenerational effects on the ovary in mice. Specifically, prenatal DEHP exposure (20 μ g/kg/day, 200 μ g/kg/day, 500 mg/kg/day, 750 mg/kg/day) dysregulates folliculogenesis, alters sex steroid hormone levels, and increases the presence of ovarian cysts in a multigenerational manner [90, 91]. Further, ancestral exposure to DEHP (20 μ g/kg/day, 200 μ g/kg/day, 500 mg/kg/day, 750 mg/kg/day) accelerates early folliculogenesis in a transgenerational manner [91]. Although studies demonstrate that phthalate exposure causes transgenerational effects on the ovary, the mechanisms causing these effects are not well understood. A few studies suggest that DEHP exposure (80 mg/kg/day, 2 g/kg, and mixtures of benzo[a]pyrene + DEHP at 5 mg/kg and 300 mg/kg, respectively and at 10 mg/kg and

600 mg/kg, respectively) causes multigenerational effects through both the ESR1 and peroxisome proliferator-activated receptor alpha [92–95]. However, further studies at human relevant doses are necessary to investigate the effects of phthalate exposure on the epigenome of the ovary, especially because the body burden of DEHP is approximately 19.17 μ g/kg as of 2015–2016 [96]. Although previous studies provide insight in to the mechanism of phthalate exposure on ovarian phenotypes, it is necessary to elucidate epigenetic mechanisms across generations.

BPA causes both multigenerational and transgenerational effects on the ovary. Prenatal exposure to BPA (20 μ g/kg/day) decreases serum testosterone levels in the F2 generation and dysregulates steroidogenic enzymes in the F2 ovaries of mice [97]. Ancestral exposure to BPA (0.5, 20, and 50 μ g/kg/day) dysregulates gene expression of ovarian apoptotic factors, oxidative stress factors, and autophagy factors in mice [98]. Interestingly, some studies have linked BPA exposure to DNA methylation across generations in females [89, 99]. Specifically perinatal exposure to BPA (10 mg/kg/BW/day) altered DNA methylation at a differentially methylated region that regulates expression of *Igf2* gene in F1 and F2 generations; however, this was found only in male mice [99]. Another study demonstrated that prenatal exposure to a mixture of BPA and phthalates (BPA 50 mg/kg BW/day, DEHP 750 mg/kg BW/day, and DBP 66 mg/kg/BW/day) promotes epigenetic transgenerational inheritance of disease. However, this study only showed that the plasticizer mixture affected the differentially methylated regions in sperm. Epigenetic analyses were not performed on the females [89]. Thus, future studies should examine the epigenetic mechanism underlying the toxic effects of BPA on the ovary using exposure levels that mimic human daily exposure (about 46.8 ng/kg/day) [100].

Vinclozolin, a fungicide used on fruits, acts as an endocrine disruptor and causes transgenerational effects [54, 101]. Prenatal vinclozolin exposure (100 mg/kg BW/day) decreases primordial follicle counts in both the F1 and F3 generations of rats at 1 year of age [101]. In addition, ancestral exposure to vinclozolin (100 mg/kg BW/day) causes small and large cysts in the ovaries and increases circulating androstenedione levels in the F3 generation [101]. The observed phenotype in the F3 generation is similar to the clinical phenotype in women with polycystic ovarian syndrome [101]. Further, vinclozolin causes differential gene expression in the F3 ovaries. These genes are associated with ovarian diseases such as polycystic ovarian syndrome. Ancestral vinclozolin exposure (100 mg/kg BW/day) also alters DNA methylated regions in promoter regions of the granulosa cells; however, the DNA methylation changes do not overlap with the promoters of the differential gene expression in the F3 generation [101]. Instead, the DNA methylation changes may influence distal gene expression through ncRNA, which may regulate the differential gene expression in the granulosa cells of the F3 ovaries [101]. Further investigations reveal that purified rat granulosa cells from the vinclozolin (100 mg/kg BW/day) exposed F3 generation have differentially expressed lncRNA and sncRNAs and that these changes contribute to the vinclozolin-induced dysregulation of the ovary [102]. Overall, these studies suggest that vinclozolin exposure (100 mg/kg BW/day) induces transgenerational epigenetic effects via ncRNA in the ovary. These studies provide a mechanistic approach to understanding vinclozolin exposure. However, the dose used in these studies is high compared to the estimated daily intake of vinclozolin, which is between 2.0–11.5 ng/kg/day [85]. Therefore, it is necessary for future studies to expand these transgenerational studies with additional epigenetic analyses using environmentally relevant levels of vinclozolin.

Epigenetic effects of endocrine disruptors on the uterus

The uterus is critical for fertility in females; it acts as an endocrine sensitive organ that facilitates both embryo implantation and parturition. Studies indicate that endocrine disruptors can affect the uterus and that these changes may lead to epigenetic and transgenerational inheritance of diseases. The prescription of DES to pregnant women is one of the best examples of multi- and transgenerational impact because it is associated with fetal endocrine disruption and adverse reproductive health outcomes in subsequent generations in humans [103]. Women who were exposed to DES as a fetus, also known as “DES daughters”, have more frequent benign reproductive tract problems, including reproductive organ dysfunction, abnormal pregnancies, structural changes of the uterus, and reduced fertility [80]. These women have an increased risk of a rare clear-cell cervicovaginal adenocarcinoma and squamous-cell and cervicovaginal carcinoma [104]. Further, these “DES daughters” report that their in utero exposure led to cancer in their daughters, the F2 generation [105]. Effects seen in this F2 generation demonstrate a multigenerational effect of prenatal DES exposure on the uterus in humans [105]. Animal studies further demonstrate multigenerational effects of DES. Specifically, prenatal DES exposure (2.5, 5, 10 $\mu\text{g}/\text{kg}/\text{day}$) decreases fertility in the F1 generation of female mice and increases the incidence of malignant reproductive tract tumors such as adenocarcinomas in the F2 generation of female mice [106]. Additional studies show that perinatal exposure to DES (1 $\mu\text{g}/\text{kg}$ BW) increases the susceptibility of uterine developmental abnormalities and cancer in both the F1 and F2 generations of female mice [107, 108].

Currently, the mechanisms explaining the multigenerational effects of DES exposure on the uterus are not fully understood. However, studies in mice suggest epigenetic alterations in DNA methylation involving hormone responsive families of genes including lactoferrin, homeobox, wnt signaling pathway, and epidermal growth factor genes are involved with the reproductive tract developmental changes in a multigenerational manner [79, 109]. Another study demonstrates that neonatal DES exposure (1 mg/kg) alters the expression of chromatin-modifying proteins, DNA methylation mediators, and DNA methylation in the adult mouse uterus, causing persistently altered epigenetic marks [110]. Further, neonatal DES exposure decreases *Dnmt* gene expression and alters DNA methylation in the mouse uterus [111]. Although these epigenetic markers are observed from neonatal exposure within the same generation, these epigenetic changes may help contribute to the multigenerational effects of DES exposure. Although some of the epigenetic mechanisms underlying the effects of DES exposure on the F3 uterus have been identified, the epigenetic mechanisms underlying DES toxicity in the F3 generation and beyond are not understood.

Another endocrine disruptor that targets the uterus is 2,3,7,8-TCDD. TCDD is a byproduct of incomplete combustion of a variety of products such as fossil fuels, wood, and industrial wastes. Studies show that TCDD exposure causes uterine dysfunction across generations [112–116]. For example, perinatal TCDD (10 $\mu\text{g}/\text{kg}$) exposure causes endometriosis-like reproductive phenotypes in F1–F4 generations of female mice, indicating both multi- and transgenerational effects [112, 113]. Further, TCDD exposure (10 $\mu\text{g}/\text{kg}$) reduces uterine progesterone responsiveness and causes subfertility [112, 114]. TCDD exposure (10 $\mu\text{g}/\text{kg}$) increases both stromal cell and epithe-

lial cell ERS2 protein expression in the F1–F3 generations and causes adenomyosis in the F3 generation [115]. Interestingly, ancestral exposure to TCDD (10 $\mu\text{g}/\text{kg}$) causes hypermethylation of *Pgr*, which is associated with the development of the endometriosis-like histological and functional phenotypes [116]. The studies above demonstrate that a single dose of TCDD at 10 $\mu\text{g}/\text{kg}$ causes transgenerational inheritance of uterine endometriosis-like phenotypes and epigenetic modification of *Pgr*, suggesting that this may be a mechanism of action. The dose 10 $\mu\text{g}/\text{kg}$ was used because it is not teratogenic or abortifacient, is below the LD_{50} for the strain, and is rapidly cleared in mice [116, 117]. However, the NHANES study shows that between 1999–2010, TCDD levels ranged between 0.4–12.1 pg/g lipid in human serum [96]. Thus, it may be likely that the 10 $\mu\text{g}/\text{kg}$ dose used in previous studies is relatively high compared to human exposure. Therefore, even though previous studies provide insight on the mechanism of action of TCDD exposure, future studies should be conducted using lower doses of TCDD.

Transgenerational and multigenerational effects of endocrine disruptors on female reproductive outcomes

Plasticizers that act as endocrine disruptors interfere with female reproductive outcomes. Some of these reproductive outcomes are due to impacts on the hypothalamus, pituitary, ovary, and uterus. For example, prenatal BPA exposure (0.5, 20, and 50 $\mu\text{g}/\text{kg}/\text{day}$) reduces the ability of mice to maintain pregnancies in a multigenerational manner and it delays the onset of puberty and compromises the ability of mice to become pregnant in a transgenerational manner [118]. BPA exposure in the diet (5 mg/kg) has been shown to interfere with brain and ovarian functions in a transgenerational manner, likely contributing to altered reproductive outcomes [61, 118]. A study on Chinese textile workers shows that in utero serum DDE levels (15 $\mu\text{g}/\text{L}$) were associated with an early menarche in the offspring [119]. Both prenatal and ancestral exposure to a mixture of phthalates (21% DEHP, 35% DEP, 15% DBP, 8% DiBP, 5% BBzP, and 15% DiNP at 20 μg , 200 mg, and 500 mg/kg/day) cause pregnancy complications in the F2 and F3 generations of mice [86]. Further, ancestral exposure to a phthalate mixture (21% DEHP, 35% DEP, 15% DBP, 8% DiBP, 5% BBzP, and 15% DiNP at 500 mg/kg/day) reduces total litter size and the percentage of dams that produce live litters in the F3 generation [86], whereas exposure to DEHP, a single phthalate, causes multi- and transgenerational effects on reproductive outcomes [91, 120]. Specifically, prenatal DEHP exposure (500 mg/kg/day) increases litter size and decreases the percentage of dams that give birth in the F2 generation [120]. Ancestral DEHP exposure (500 mg/kg/day) accelerates the onset of puberty and reproductive senescence in the F3 generation of female mice [90, 120]. The ability of phthalates to interfere with ovarian functions may contribute to these altered reproductive outcomes [86, 90, 91, 120, 121].

Recently, a study was published on the reproductive and hormone-related outcomes in women over the age of 18 that were third generation exposed to DES. This study shows that in the third generation, women had an increased risk of irregular menses, amenorrhea, pre-term delivery, and ectopic pregnancies. These changes were more apparent in women whose mother was affected by DES-associated vaginal epithelial changes [122].

Summary/Conclusion

Overall, the literature shows that several endocrine disrupting chemicals cause reproductive dysfunction in females in a multigenerational and transgenerational manner and that some of these effects are due to epigenetic changes. Epidemiological data show that exposure to endocrine disruptors is associated with adverse ovarian and uterine health outcomes in women across generations [78, 105, 119]. Experimental data demonstrate that endocrine disruptors cause female reproductive abnormalities in the hypothalamus, ovary, and uterus in multigenerational and transgenerational manners [61, 82, 86, 90, 91, 106, 120, 123]. Generally, the consensus is that epigenetic changes are induced by chemical exposures and are inherited through the germline, thus causing transgenerational phenotypes in reproductive functions in the generation that was not directly exposed to the endocrine disruptor. However, it is critical that future studies continue to investigate the epigenetic basis of transgenerational inheritance and demonstrate that the epigenetic changes are inherited through the germline. Specifically, it would be beneficial to map a time course of endocrine disruptor exposure in the developing gonad and how the exposure directly influences the epigenetic machinery for each generation. Such information could provide insight into why some studies, but not all studies, observe effects of endocrine disruption on reproduction and the epigenome. Additionally, determining whether histone modifications at a certain chromosomal location and/or DNA methylation profiles are conserved throughout generations would provide potential targets of interest for future mammalian studies on environmental endocrine disruptors. Finally, it is imperative that future studies use doses that are environmentally relevant to accurately assess the reproductive and epigenetic effects on the body. Endocrine disruptors do not follow a monotonic dose response, and therefore different doses will produce different effects on the body. It is crucial to fill the gap in knowledge about how endocrine disruptors affect the epigenome so that potential interventions can be developed and used to stop endocrine disruption of female reproductive health.

Acknowledgment

We thank Catheryne (Katie) Chiang for her exemplary illustrations in this review.

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