Environmentally Induced Epigenetic Transgenerational Inheritance of Female Reproductive Pathology

Michael K Skinner and Eric E Nilsson, Center for Reproductive Biology, School of Biological Sciences, Washington State University, Pullman, WA, United States

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Abstract

Nearly all environmental factors from diet to toxicants can impact biology and reproduction. These effects can impact the epigenetic programming of the germline (eggs or sperm) epigenetics to promote the epigenetic transgenerational inheritance of pathology and phenotypes in subsequent generations. One of the most sensitive organ systems and biological processes is reproduction. The impacts of environmental exposures on female reproductive biology through epigenetic transgenerational inheritance is reviewed. The organ systems impacted include the ovary, female reproductive tract, mammary gland, and reproductive behavior.

Glossary

DNA methylation An epigenetic process in which a methyl group attaches to a cytosine molecule that is next to a guanine on the DNA strand.

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

Polycystic ovarian syndrome (PCOS) An individual having oligo/anovulation, hyperandrogenism, or polycystic ovaries on an ultrasound. In humans, PCOS is characterized as an individual having two of the three above conditions.

Primary ovary insufficiency (POI) A condition characterized by marked follicular loss within the ovaries and menopause before the age of 40 in humans.

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

Key Points

- Environmentally Induced Epigenetic Transgenerational Inheritance of Female Reproductive Pathology.

Introduction

Epigenetic mechanisms can mediate changes in an organism's phenotype or gene expression patterns that occur in response to environmental factors. Epigenetic processes include DNA methylation, modifications to histone proteins, expression of non-coding RNA, and changes to chromatin structure. Ancestral exposure to a variety of environmental factors such as toxicants or abnormal nutrition, has been shown to promote changes in transgenerational gene expression and disease frequency (Skinner, 2014; Anway *et al.*, 2005; Nilsson *et al.*, 2022). This concept is known epigenetic transgenerational inheritance. Transgenerational phenomena are inherited changes that do not involve any form of continued direct environmental factor exposure, while intergenerational inheritance involves direct exposures to the animal, developing embryo, or developing gametes within a developing embryo. Transgenerational epigenetic inheritance requires that epigenetic information or epigenetic changes are present in germ cells (i.e., sperm or eggs), as it is through germ cells that inheritance occurs (Anway *et al.*, 2005).

There are some periods of an organism's development that are sensitive to environmental impacts, during which exposure to environmental factors can change the epigenetic landscape around an organism's DNA. In mammals one such sensitive window is during the period of gonadal sex determination, at which time extensive epigenetic modifications are already occurring in the developing germ cells (Messerschmidt *et al.*, 2014; Ben Maamar *et al.*, 2021c). Toxicants administered to a pregnant F0 generation female at this time directly expose the developing pups (F1 generation) to the toxicant, as well as exposing the developing gametes within those pups, which will become the F2 generation. Thus, a transgenerational effect can only be seen in the F3 generation animals (great-grand-offspring) and beyond because those animals would not be directly exposed to any toxicants. 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, and examination of the F2 generation (grand-offspring) is required to demonstrate a transgenerational phenomenon (Skinner, 2008), **Fig. 1**.

A germline with an altered epigenome following fertilization impacts the epigenetic programming of the embryonic stem cells, which then can impact the epigenetic programming of all subsequent somatic cell types in the developing organism from neurons to female reproductive organ cell types (Ben Maamar *et al.*, 2021c). In the female this includes reproductive organs such as the ovary, female reproductive tract, mammary gland, and reproductive behavior. One of the most sensitive female reproductive tissues is the ovary.

Ovarian diseases affect many individuals throughout the world. The two most common types of ovarian diseases are polycystic ovarian syndrome (PCOS) and primary ovarian insufficiency (POI). Polycystic ovarian syndrome affects 12–21% of women of reproductive age (March *et al.*, 2010). PCOS in humans is characterized by a combination of different ovarian abnormalities including oligo/anovulation, hyperandrogenism, and polycystic ovaries on an ultrasound (Boyle and Teede, 2012). Primary ovary insufficiency is a condition characterized by oocyte loss within the ovaries and menopause before the age of forty in humans (Laven, 2016).

Transgenerational epigenetic effects caused by different factors, such as environmental pollutants, can be evaluated both in terms of molecular epigenetic changes induced within the cells of descendants, and in characterizing observable phenotypic changes in descendants, such as increases in disease frequencies.

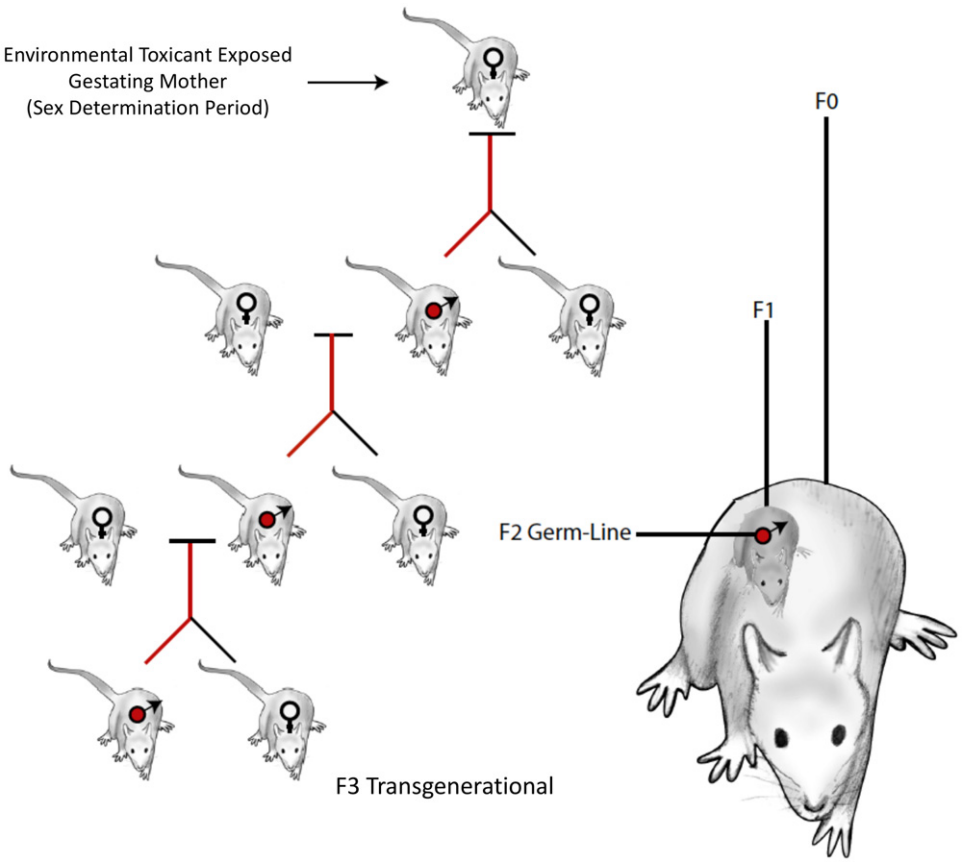

Fig. 1 Rat lineage example showing an F0 generation pregnant animal exposed to a toxicant, and illustrating that the multigenerational F1 and F2 generations were also directly exposed, while the transgenerational F3 generation had no direct exposure. Modified from Skinner (2008).

Epigenetic Transgenerational Impacts on Female Reproductive Pathology

Several environmental toxicants have been studied in model animal species for the potential epigenetic transgenerational effects they may have on female reproduction.

Vinclozolin is a known antiandrogenic fungicide that is commonly used on fruits and vegetables, specifically wine grapes and fruit in the United States (Anway and Skinner, 2006). Following exposure of F0 generation pregnant rats to vinclozolin, the directly exposed F1 generation females at one year of age showed a significant increase in small ovarian cysts compared to controls. This resembles PCOS in humans. These F1 generation ovaries also had a significant decrease in primordial and developing follicles, reflecting a reduction in the total number of oocytes in the ovary. Such decreases in the primordial follicle pool size are similar to what is seen in POI in humans and may lead to lower fertility. In the non-exposed F3 generation of vinclozolin lineage animals, the ovaries showed an increased incidence of both small and large ovarian cysts, as well as a significant decrease in oocyte numbers (Nilsson *et al.*, 2012). This F3 generation phenotype indicates epigenetic transgenerational inheritance of ovarian disease. Ancestral exposure to vinclozolin in rats has also resulted in an increase in uterine bleeding events late in pregnancy in the F1–F3 generations (Nilsson *et al.*, 2008). Granulosa cells isolated from antral follicles of the transgenerational F3 generation vinclozolin lineage ovaries showed epigenetic differences compared to controls. Forty-three genes showed changes in DNA methylation in promoter regions, some of which are known to be correlated with ovarian disease (Nilsson *et al.*, 2012). In addition to ovarian disease, female pubertal onset was effected, mammary tumor frequency, and reproductive behavior has been sown to be altered (Skinner *et al.*, 2014; Nilsson *et al.*, 2018, 2022), **Table 1**.

Permethrin is an insecticide used to treat both animals and crops (Li *et al.*, 2016). DEET (N,N-diethyl-meta-toluamide) is the most common active ingredient in insect repellents and is commonly applied topically to the skin (Osimitz and Grothaus, 1995). Following exposure of F0 generation gestating female rats to a mixture of permethrin and DEET, the directly exposed F1 generation females showed a decrease in primordial follicles, indicating a lower follicle pool size. The non-exposed F3 generation rats also experienced a decrease in primordial follicle numbers, and an increase in both small and large cyst frequency as compared to F3 non-exposed vehicle control rats (Nilsson *et al.*, 2012) (See **Table 1**). In addition, the incidence of F3 generation females with an altered age of puberty was increased (Manikkam *et al.*, 2012b). Multiple reproductive diseases and others were also observed (Nilsson *et al.*, 2022), **Table 1**.

Dichlorodiphenyltrichloroethane (DDT) is an environmentally persistent insecticide that was heavily used in the USA until its use was banned 1972. Exposure to DDT may occur when consuming meat, fish, or dairy products (United States Center for Disease Control and Prevention DDT Factsheet). Following the treatment of pregnant F0 generation rats with DDT, the transgenerational F3 generation rats had an increase in incidence of small and large cysts compared to controls (Skinner *et al.*, 2013; Nilsson *et al.*, 2018). In addition, DDT promoted transgenerational mammary tumors (Ben Maamar *et al.*, 2020a), **Table 1**.

Methoxychlor is an insecticide used on agricultural crops and in animal feed (ATSDR, 2002). In rat studies of F0 generation gestating females exposed to methoxychlor, the resulting F3 generation animals showed a transgenerational increase in polycystic ovary incidence (Manikkam *et al.*, 2014). In addition, transgenerational mammary tumors and puberty abnormalities were observed, **Table 1**.

Bisphenol-A (BPA), Dibutyl phthalate (DBP), and di(2-ethylhexyl)phthalate (DEHP) are components of plastics that are environmental toxicants that humans are exposed to on a regular basis (Lorz *et al.*, 2002). After F0 generation pregnant female rats were exposed to a mixture of BPA, DBP and DEHP, the F1 generation females were found to have fewer oocytes in their ovaries, and an

Table 1 Environmental (toxicants) impacts on transgenerational female reproductive pathology

Environmental toxicant	*Transgenerational effect*	*Reference*
Vinclozolin	Ovarian oocyte decrease, cyst increase, granulosa cell gene expression change, uterine bleeding, pubertal onset, sexual selection, mammary tumors	(Nilsson *et al.*, 2012, 2008, 2018; Skinner *et al.*, 2014; Ben Maamar *et al.*, 2020a)
Pesticides (Permethrin and DEET)	Ovarian oocyte decrease, cyst increase, altered pubertal age	(Nilsson *et al.*, 2012; Manikkam *et al.*, 2012b)
DDT	Ovarian cyst increase, mammary tumors	(Skinner *et al.*, 2013; Ben Maamar *et al.*, 2020a)
Methoxychlor	Ovarian cyst increase, puberty, mammary tumors	(Manikkam *et al.*, 2014; Ben Maamar *et al.*, 2020a)
Plastics mix	Ovarian oocyte decrease, cyst increase. Altered pubertal age	(Nilsson *et al.*, 2012; Manikkam *et al.*, 2013; Thorson *et al.*, 2021)
DEHP (mice)	Ovarian oocyte decrease, reduced embryo viability	(Pocar *et al.*, 2017)
BPA (mice)	Altered ovarian gene expression	(Berger *et al.*, 2016)
Dioxin	Ovarian oocyte decrease, cyst increase. Altered pubertal age (rats). Reduced pregnancy rates, increased pre-term birth, endometriosis (mice)	(Nilsson *et al.*, 2012; Bruner-Tran and Osteen, 2011; Ben Maamar *et al.*, 2021b)
Jet Fuel	Ovarian oocyte decrease, cyst increase. Altered pubertal age	(Nilsson *et al.*, 2012; Tracey *et al.*, 2013; Ben Maamar *et al.*, 2020b)
Glyphosate	Ovarian decrease, pubertal onset, mammary tumors	(Ben Maamar *et al.*, 2021a; Kubsad *et al.*, 2019)

increase in small and large cysts. In the F3 generation, there was a transgenerational decrease in oocytes, an increase in small and large cysts, and an increased incidence of females with an altered age of puberty (Nilsson *et al.*, 2012; Manikkam *et al.*, 2013). In a mouse study investigating BPA alone, after F0 generation pregnant animals were exposed it was found that the F3 generation had ovaries with altered expression of hormone receptor, steroidogenic, apoptotic, and anti-oxidant genes (Berger *et al.*, 2016). In a mouse study investigating DEHP alone, following exposure of the pregnant F0 generation animals, DEHP was found to cause reduced oocyte number and reduced embryo viability over several generations of female mice (Pocar *et al.*, 2017), **Table 1**. Epigenetic associations with female reproductive disease has also been observed (Thorson *et al.*, 2021).

Dioxin is an organic pollutant that is a byproduct of chemical manufacturing processes. It is a reproductive toxicant and can alter hormone release, and cause developmental issues or cancer when direct exposure occurs (WHO, 2010). Following the treatment of F0 generation gestating female rats with dioxin, the F1 generation treated lineage ovaries were found to have a significant decrease in oocyte numbers. The F3 generation animals also experienced a significant transgenerational decrease in oocyte pool size, as well as a significant increase in the incidence of small cysts, and a decrease in the age of puberty (Nilsson *et al.*, 2012). In mice exposure of F0 generation pregnant animals to dioxin resulted in a transgenerational reduction in pregnancy rates seen in the F3 and F4 generations, as well as a transgenerational increase in the incidence of pre-term birth, and increased uterine pathologies resembling endometriosis (Bruner-Tran *et al.*, 2016; Bruner-Tran and Osteen, 2011), **Table 1**. Epigenetic transgenerational biomarkers for dioxin induced reproductive disease has been observed (Ben Maamar *et al.*, 2021b).

JP-8 is a hydrocarbon mixture used as fuel for jet aircraft. It has been used as a carrier for pesticides and is commonly applied for dust control on gravel roads (Witzmann *et al.*, 2003). After pregnant rats were exposed to jet fuel, the F1 generation directly exposed animals experienced a decrease in oocyte numbers, as well as an increase in small ovarian cysts. The F3 generation showed a transgenerational decrease in oocyte numbers, as well as an increase in the incidence of both small and large cysts, and a decrease in the age of puberty (Nilsson *et al.*, 2012; Tracey *et al.*, 2013), **Table 1**. Epigenetic transgenerational biomarkers for jet fuel induced reproductive disease has been observed (Ben Maamar *et al.*, 2020b).

A more recent environmental toxicant exposure used is glyphosate (i.e., Roundup, Monsanto) with an F0 generation female during the gonadal sex determination period (Ben Maamar *et al.*, 2021a; Kubsad *et al.*, 2019). The F0 generation female offspring F1 generation was bred to the F2 and F3 generations for pathology and epigenetic analysis. The F3 transgenerational generation were assessed for reproductive pathologies. High levels of ovarian disease, mammary tumors, pubertal development abnormalities, and parturition abnormalities were observed in the glyphosate transgenerational lineage females (Kubsad *et al.*, 2019), **Table 1**. Epigenetic associations were identified for this female reproductive disease abnormalities (Ben Maamar *et al.*, 2021a). Currently glyphosate is one of our highest toxicant exposures through the food we eat.

As can be seen above, epigenetic transgenerational inheritance of female reproductive disease susceptibility can be induced by ancestral exposure to the 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 descendants of F0 generation gestating females that had been exposed to either vinclozolin, jet fuel hydrocarbons, dioxin, a plastics mixture, or a pesticide mixture (Manikkam *et al.*, 2012a). Interestingly, the sites of changes in DNA methylation, known as differentially methylated regions (DMR), formed a unique pattern with each different ancestral toxicant exposure. No transgenerational DMR was common to all the exposures. This shows that there are molecular epigenetic changes that correlate with inherited transgenerational phenotypes seen with each of these toxicant exposures. This also suggests that DNA 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 whether they have an increased susceptibility to the correlated diseases. More recently this study was expanded to all the exposures discussed and all the disease conditions identified (Beck *et al.*, 2022). This systems biology approach identified exposure specific and disease specific epigenetic biomarkers that demonstrate subsets of epigenetic sites and associated genes within a larger number of sites are sufficient to promote reproductive disease (Beck *et al.*, 2022). Therefore, the role of environmental epigenetics in the etiology of reproductive disease is being elucidated.

Conclusion

Studies in rodents have shown that environmental toxicants can induce epigenetic changes that can be inherited and lead to an increased incidence of reproductive diseases in females. Care must be taken when interpreting these data since the manifestations of rodent ovarian and reproductive diseases are different from those in humans. However, it is interesting to note that the incidence of human ovarian diseases is now as high as 20% (Sirmans and Pate, 2013), and ancestral exposures to environmental toxicants must be considered as one underlying cause (Nilsson *et al.*, 2022).

Exposure to different environmental factors has the potential to alter the epigenome of an individual, as well as transgenerationally in subsequent non-exposed generations, which induces changes in reproductive disease frequency. Therefore, environmental exposures could contribute to the incidence of reproductive abnormalities in the current human population. The transgenerational induction of disease susceptibility within the population is likely a factor in our high pathology rates within the population. Epigenetic profiling of individuals may be able to provide biomarkers of ancestral exposure, and so predict their susceptibility to specific diseases, and allow preventative medicine.

References

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

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.

Atsdr, 2002. Toxicological profile for methoxychlor. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. ATSDR.

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

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., Thorson, J.L.M., Beck, D., Skinner, M.K., 2021b. Transgenerational disease specific epigenetic sperm biomarkers after ancestral exposure to dioxin. Environmental Research 192, 110279.

Ben Maamar, M., King, S.E., Nilsson, E., Beck, D., Skinner, M.K., 2020a. Epigenetic transgenerational inheritance of parent-of-origin allelic transmission of outcross pathology and sperm epimutations. Developmental Biology 458, 106–119.

Ben Maamar, M., Nilsson, E., Thorson, J.L.M., Beck, D., Skinner, M.K., 2020b. Epigenome-wide association study for transgenerational disease sperm epimutation biomarkers following ancestral exposure to jet fuel hydrocarbons. Reproductive Toxicology 98, 61–74.

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

Berger, A., Ziv-Gal, A., Cudiamat, J., *et al.*, 2016. The effects of in utero bisphenol A exposure on the ovaries in multiple generations of mice. Reproductive Toxicology 60, 39–52.

Boyle, J., Teede, H.J., 2012. Polycystic ovary syndrome - an update. Australian Family Physician 41, 752–756.

Bruner-Tran, K.L., Osteen, K.G., 2011. Developmental exposure to TCDD reduces fertility and negatively affects pregnancy outcomes across multiple generations. Reproductive Toxicology 31, 344–350.

Bruner-Tran, K.L., Duleba, A.J., Taylor, H.S., Osteen, K.G., 2016. Developmental toxicant exposure is associated with transgenerational adenomyosis in a murine model. Biology of Reproduction 95, 73.

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. Scientific Reports 9, 6372.

Laven, J.S., 2016. Primary ovarian insufficiency. Seminars in Reproductive Medicine 34, 230–234.

Li, W., Morgan, M.K., Graham, S.E., Starr, J.M., 2016. Measurement of pyrethroids and their environmental degradation products in fresh fruits and vegetables using a modification of the quick easy cheap effective rugged safe (QuEChERS) method. Talanta 151, 42–50.

Lorz, P.M., Towae, F.K., Enke, W., *et al.*, 2002. Phthalic acid and derivatives. Ullmann's Encyclopedia of Industrial Chemistry. Weinheim: Wiley-VCH.

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. Reproductive Toxicology 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.

March, W.A., Moore, V.M., Willson, K.J., *et al.*, 2010. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Human Reproduction 25, 544–551.

Messerschmidt, D.M., Knowles, B.B., Solter, D., 2014. DNA methylation dynamics during epigenetic reprogramming in the germline and preimplantation embryos. Genes & Development 28, 812–828.

Nilsson, E.E., Anway, M.D., Stanfield, J., Skinner, M.K., 2008. Transgenerational epigenetic effects of the endocrine disruptor vinclozolin on pregnancies and female adult onset disease. Reproduction 135, 713–721.

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

Nilsson, E., Klukovich, R., Sadler-Riggleman, I., *et al.*, 2018. Environmental toxicant induced epigenetic transgenerational inheritance of ovarian pathology and granulosa cell epigenome and transcriptome alterations: Ancestral origins of polycystic ovarian syndrome and primary ovarian insufiency. Epigenetics 13, 875–895.

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

Osimitz, T.G., Grothaus, R.H., 1995. The present safety assessment of deet. Journal of the American Mosquito Control Association 11, 274–278.

Pocar, P., Fiandanese, N., Berrini, A., Secchi, C., Borromeo, V., 2017. Maternal exposure to di(2-ethylhexyl)phthalate (DEHP) promotes the transgenerational inheritance of adult-onset reproductive dysfunctions through the female germline in mice. Toxicology and Applied Pharmacology 322, 113–121.

Sirmans, S.M., Pate, K.A., 2013. Epidemiology, diagnosis, and management of polycystic ovary syndrome. Clinical Epidemiology 6, 1–13.

Skinner, M.K., 2008. What is an epigenetic transgenerational phenotype? F3 or F2. Reproductive Toxicology 25, 2–6.

Skinner, M.K., 2014. Endocrine disruptor induction of epigenetic transgenerational inheritance of disease. Molecular and Cellular Endocrinology 398, 4–12.

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

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.E., Skinner, M.K., 2021. Ancestral plastics exposure induces transgenerational disease-specific sperm epigenome-wide association biomarkers. Environmental Epigenetics 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. Reproductive Toxicology 36, 104–116.

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.