Spring 2024 – Systems Biology of Reproduction
Lecture Outline – Epigenetics and Transgenerational Reproductive Disease
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
March 5, 2024
Week 9

Epigenetics and Transgenerational Reproductive Disease

- Environmental Induced Pathology
- Transgenerational Disease Phenotype
- Compound Specificity
- Epigenetic Mechanism
- Epimutations and Exposure Specificity
- Transgenerational Transcriptome
- Transgenerational Testis Disease
- Transgenerational Ovary Disease
- Broader Impact

REQUIRED READING

Ben Maamar M, Nilsson EE, Skinner MK. Epigenetic transgenerational inheritance, gametogenesis and germline development. Biol Reprod. 2021 Sep 14;105(3):570-592.

Wang HD, Allard P. Challenging dogmas: How transgenerational epigenetics reshapes our views on life. J Exp Zool A Ecol Integr Physiol. 2022 Jan;337(1):70-74.

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Epigenetic transgenerational inheritance, gametogenesis and germline development†

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Abstract

One of the most important developing cell types in any biological system is the gamete (sperm and egg). The transmission of phenotypes and optimally adapted physiology to subsequent generations is in large part controlled by gametogenesis. In contrast to genetics, the environment actively regulates epigenetics to impact the physiology and phenotype of cellular and biological systems. The integration of epigenetics and genetics is critical for all developmental biology systems at the cellular and organism level. The current review is focused on the role of epigenetics during gametogenesis for both the spermatogenesis system in the male and oogenesis system in the female. The developmental stages from the initial primordial germ cell through gametogenesis to the mature sperm and egg are presented. How environmental factors can influence the epigenetics of gametogenesis to impact the epigenetic transgenerational inheritance of phenotypic and physiological change in subsequent generations is reviewed.

Summary sentence

How environmental factors can influence the epigenetics of gametogenesis to impact the epigenetic transgenerational inheritance of phenotypic and physiological change in subsequent generations is reviewed.

Key words: Gametogenesis, Spermatogenesis, Oogenesis, PGCs, Epigenetics, Transgenerational, Review.

Introduction

The germ line is an enduring link between all generations of a species. After the fertilization of the oocyte by the sperm, a totipotent zygote will give rise to all cell lineages of the organism, including the germ line itself. The primordial germ cells (PGCs) are the precursor pluripotent stem cells for the sperm and egg. They are established during the perigastrulation epiblast stage of the mammalian embryo. The PGCs specification is regulated by a unique and complex gene network induced by signals from extra-embryonic tissues [1]. In the gonads, these PGCs will differentiate into the male prospermatogonia or female oogonia in response to Sertoli or granulosa cell signaling. The prospermatogonia continue in the gametogenesis process and undergo spermatogenesis to develop into the mature sperm. The oogonia continue into the gametogenesis process and undergo oogenesis to develop into the mature oocyte. Therefore, gametogenesis can be seen as a crucial first step for the perpetuation of the mammalian life cycle [2].

The crucial aspect of gametogenesis is the production of genetically and epigenetically competent gametes, which are necessary

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for normal fertilization and the organism's development. Epigenetics is defined as the factors and processes around DNA that require genome activity independent of DNA sequence, and are mitotically stable. The components include DNA methylation, histone modifications, and non-coding RNA chromatin structure that regulate gene activity independent of DNA sequence [3]. Maternal and paternal gametes display genomic imprinted differences due to DNA methylation and other epigenetic modifications established in the germ line during gametogenesis, but this is only a small component of the epigenome and its regulation of biology.

Interestingly, previous studies have shown that epigenetic modifications can occur during gametogenesis under the influence of environmental factors (stress, diet, pollutants, etc.), which can lead to phenotypical defects in the individuals exposed and in the subsequent generations through the germline. This non-genetic form of inheritance is termed epigenetic transgenerational inheritance and is mediated through epigenetic alterations (i.e. epimutations) in the sperm or egg.

The current review presents the molecular basis of germ cell development (i.e. gametogenesis), and also how the environment can induce stable epimutations and modified phenotypes through the transgenerational inheritance phenomenon.

Mammalian gametogenesis and primordial germ cell development

Primordial germ cell specification

The totipotent stem cells derived from the zygote are the product of fertilization of the oocyte by the sperm, which gives rise to all cell lineages of an organism, and the germline itself. Thus, the specification of primordial germ cells (PGCs) is a pivotal first step for the acquisition of the germline pluripotent cell and the continuation of the mammalian life cycle [1]. In metazoans, two different processes form the germline in the male and female, giving rise to sperm and oocytes. *Caenorhabditis elegans* and *Drosophila melanogaster* have been used to describe the mechanism for PGC specification in invertebrates, and Zebrafish and *Xenopus* in non-mammalian vertebrates [4–7]. At the onset of development, preformation of germ plasm segregates the germ and the soma. The germ plasm is comprised of RNA, proteins, and organelles that are grouped in a specific location within the oocytes, then allocated to a few cells in the germline of the developing embryo [8]. In this instance, the germline is always differentiated from somatic cells across generations. Alternatively, in mammals, the germline is induced within a population of pluripotent cells. The ectopic expression of germline genes in the soma can be tumorigenic [9], however, specification failure in the germline is a reproductive dead end. This specification process requires a precise orchestration to ensure a timely restriction from the soma. Somatic cells not allocated to the germline will undergo differentiation and perish, whereas the germline has the ability of establishing a new organism in the next generation [10]. The primordial germ cells are specified during early embryonic development. Bone morphogenic protein (BMP) signaling is indispensable for PGC specification, and targeted disruption of *Bmp2*, *Bmp4*, *Bmp8b*, or BMP signaling transducers *Smad1*, *Smad4*, *Smad5* or *Alk2*, all demonstrate loss or reduced numbers of PGC [11–16].

The first phase of gametogenesis happens in early embryogenesis, during the formation and migration of PGCs into the gonadal ridge [17, 18]. Mouse models have been primarily used to study the mammalian germ cell development. In the early post-implantation embryo epiblast, PGCs specification is initiated. At mouse embryonic day E6.25 in some pluripotent epiblast cells, bone morphogenetic protein (BMP) and WNT signals from extra-embryonic tissues to induce the expression of a key regulator of PGC fate: the PR domain zinc-finger protein 1 (PRDM1, also known as BLIMP1) [2, 19]. Two other factors are upregulated next, PRDM14 and the transcription factor AP2γ (encoded by Tfap2c) [20, 21]. The germ cell fate is then induced by the transcription factor network formed by PRDM1, PRDM14, and AP2γ [22–24]. This tripartite network suppresses somatic gene expression such as *Hoxa1*, *Hoxb1*, *Lim1*, *Evx1*, *Fgf8*, and *Snail* genes, while initiating the germ cell transcriptional program but also setting off a genome-wide epigenetic reprogramming [13, 20, 22, 25–28]. Interestingly, Blimp1 and Prdm14 have distinct binding patterns relative to promoters, whereas Blimp1 is important for the repression of almost all genes usually downregulated in PGCs, as well as for the restoration of pluripotency and epigenetic reprogramming (Figure 1) [29]. The restoration of pluripotency and epigenetic reprogramming are regulated by Prdm14, independently from Blimp1, that defines a novel genetic pathway with strict specificity to the germ cell lineage [30]. In mice, the knockout (KO) of Blimp1, Prdm14, or Ap2γ result in PGCs specification defects highlighting the fact that these three factors are dominant coordinators of the transcriptional program for the establishment of the germ cell fate. In addition, the concomitant overexpression of these three factors in cells *in vitro* induces mouse germ cell formation in the absence of cytokines [22] shows again the importance of these three transcription factors. After embryonic day 7 (E7) in the mouse, the PGCs are then specified and express PGC-characteristic markers, such as stage-specific embryonic antigen 1 (SSEA1) or developmental pluripotency associated 3 (DPPA3 or STELLA) [31–34]. The expression of several pluripotent genes is also maintained in the PGCs such as *Nanog*, *Oct4* and SRY (sex-determining region-Y) [35–38]. Sybirna and collaborators recently revealed a crucial role for PRDM14 in human germ cell fate, where a loss of function affects the efficiency of specification and results in an aberrant hPGCLC transcriptome. Moreover, their study showed that PRDM14 targets are not conserved between mouse and human, which highlights the evolutionary divergence in the molecular network for PGC specification [39].

Although, most studies have been conducted in mice, recent findings in non-rodent mammals have highlighted the similarities and differences between species. In humans, the PGC formation occurs in the third week of gestation. In vitro studies of human PGCs have shown that these cells originate from mesodermal precursor cells, and BMP and WNT signaling pathways are also essential for specification [1, 40, 41]. In contrast to mice, human PGCs lack Sox2 expression. Therefore, the species differences between human and mice PGC transcriptional network may be explained by the differences in either pluripotency circuitry or embryonic origin [40].

Primordial germ cell migration

Just after their specification, between E7.5 and E10.5, while proliferating the PGCs migrate through the hindgut and genital ridge then into the developing gonads to differentiate into gametes [27, 42, 43]. Two germ cell–soma signaling pathways cKIT-STEEL and SDF-CXCR4 are required for the normal migration of PGCs. In mice, germ cells express c-KIT, whereas STEEL is expressed in the somatic cells lining the route to the gonad. The interaction between c-KIT and STEEL is fundamental for PGC proliferation, survival, and migration from the primitive streak to the genital ridge

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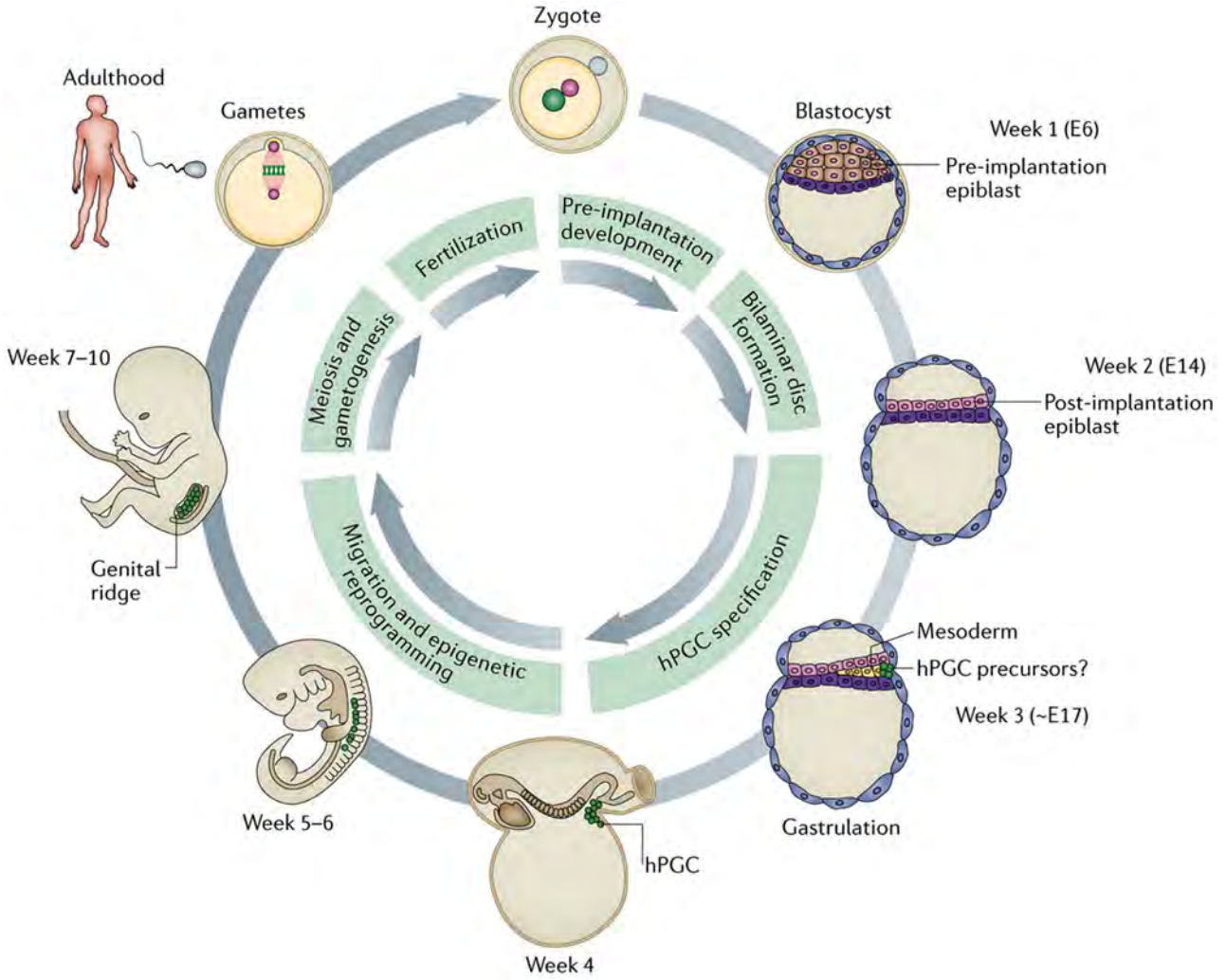

Figure 1. Human germline development. Just after fertilization, a zygote is formed. At week 1, the blastocyst develops and contains pluripotent epiblast cells, which will give rise to all lineages in the embryo, including the germ line. At week 2, the blastocyst implants into the uterine wall. The human primordial germ cells (hPGCs) are probably specified around the time of gastrulation around week 3. At week 4, the hPGCs are localized near the yolk sac wall close to the allantois. After that stage, the hPGCs migrate through the hindgut to the developing genital ridges. At this developmental stage, the migrating hPGCs go through a genome-wide epigenetic reprogramming, including global DNA demethylation, to erase imprints and other somatic epigenetic marks. During the fetus development and adult life, the germ line will undergo meiosis and gametogenesis to differentiate into sperm and eggs. At the same time, the genome is remethylated and acquires appropriate epigenetic signatures for the generation of a totipotent zygote upon fertilization (modified from [1]).

[44–48]. Sterility because of a lack of spermatogonial stem cells and thus differentiated germ cells has been observed in homozygous *cKit* and *Steel* mutant mice [46, 49–51]. The chemoattractant stromal cell-derived factor 1 (SDF-1) expressed at the genital ridges in the surrounding mesenchyme also facilitates the directional PGC migration. SDF-1 is detected by its receptor C-X-C chemokine receptor type 4 (CXCR4) expressed on the surface of PGCs [47]. A knockdown of the activity of CXCR4b and of the SDF-1a ligand has been shown to result in severe PGC migration defects such as very few PGCs reaching the genital ridge [52]. Alternatively, the migration of PGCs can be redirected toward sites of ectopically expressed SDF-1a [52–54]. This ectopic expression of SDF-1 can account for the development of some extra-gonadal cell tumors in humans [27].

During their migration, PGCs continue to proliferate and reach 500 cells in each fetal gonad at E10.5 in the mouse [55]. At this stage, the PGC differentiate into oogonia in females or gonocytes (i.e. prospermatogonia) in males. To form germline cysts, between E10.5 and E14.5, the fetal germ cells undergo five additional mitotic divisions with incomplete cytokinesis [56]. These cysts cluster together and will form germ cell nests in both female and male fetal gonads [55–57]. These germ cell nests will then resolve and generate the primary oocytes or prospermatogonia in the differentiated female and male gonads, respectively.

Primordial germ cells and epigenetics

DNA methylation. The first DNA methylation erasure period is incomplete and happens in the early embryo, leaving paternally and maternally imprinted genes intact and in all somatic lineages subsequently derived. The second DNA methylation erasure period is more comprehensive and occurs during the germline specification. The function of the DNA methylation erasure is to generate in the case of the embryo a totipotent stem cell population, and for PGCs a pluripotent stem cell population. However, despite these two

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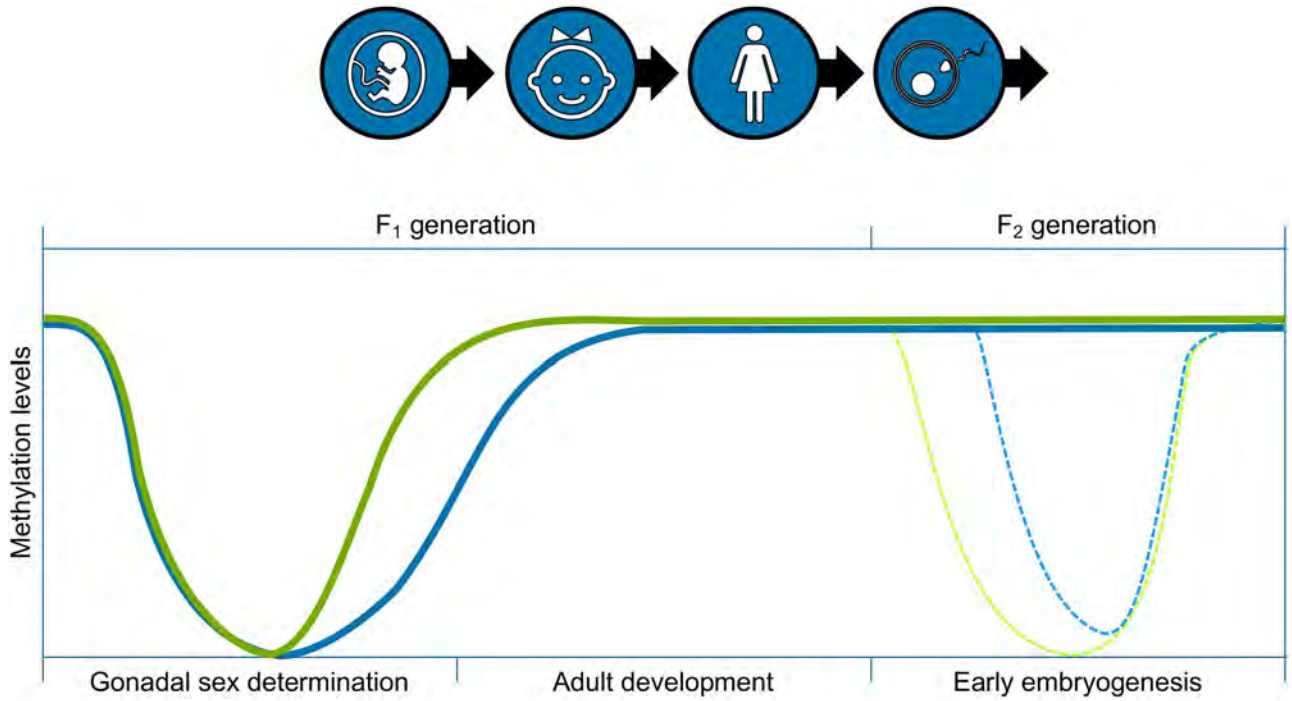

Figure 2. Epigenetic reprogramming (DNA methylation erasure) during primordial germ cell development at gonadal sex determination and following fertilization in the early embryo (modified from [283]).

epigenetic methylation erasure processes, epigenetic information can be passed down to the offspring, similar to imprinted genes, even though the mechanisms behind this process remain to be elucidated (Figure 2).

Upon specification, during the rapid proliferation of PGCs, the first DNA methylation erasure happens and consists of a passive DNA methylation between E6.5 and E10.5 in the mouse, which results from repressing de novo DNA methyltransferases DNMT3a/b [58–62]. Still, the maintenance of DNA methyltransferase 1 (DNMT1) prevents the dilution of DNA methylation modifications on parentally imprinted regions and meiotic gene promoters [63]. Moreover, a loss of Dnmt1 in PGCs results in a premature meiotic entry in females and precocious differentiation in prospermatogonia in males, and causes infertility [63].

Further demethylation erasure takes place between E10.5 and E12.5 in the mouse, while the PGCs migrate to the genital ridge and begin sex determination. At this period, DNA methylation is at its lowest level due to enzymatic activity from TET1 and TET2 removing DNA methylation [64–66]. During this DNA demethylation erasure window, the maternally and paternally imprinted loci and resistant promoter regions are erased [65, 67]. Interestingly, several studies have demonstrated that a loss of TET1 and 2 in the germ cells does not impact infertility, and few loci showed altered epigenetic states [65, 68–70]. Despite the near complete DNA demethylation erasure process, some genome loci remain methylated in human and mouse PGCs and are referred as 'escapees' [58, 64, 71, 72]. In both species, these escapees have been shown to be associated with retrotransposable elements [58, 64, 71–73], subtelomeric regions [71] and pericentrometric satellite repeats [72] also display these escapees.

The PGCs extensive epigenetic reprogramming includes a genome-wide loss of approximately 90% of 5-methylcytosine (5mC) [58, 64, 65, 68, 71, 74–77]. Even though the underlying molecular mechanisms of this process have until recently remained unclear, a set of germline reprogramming responsive (GRR) genes is crucial for the correct progression of PGC development and gametogenesis. These genes show unique promoter sequence characteristics, with high levels of both 5mC and 5-hydroxymethylcytosine (5hmC). These genes are targets of TET1, the ten eleven translocation (TET) enzymes, which oxidize 5mCs and promote locus-specific reversal of DNA methylation [78]. The PRC1 is the canonical polycomb repressive complex PRC1, which promotes compact local chromatin structures and longer-range chromatin interactions [79]. The loss of DNA methylation combined with PRC1 repression is uniquely required for GRR gene activation. In this epigenetically poised state, TET1 is required to potentiate a full and efficient activation. TET1 seems to be especially important in female PGCs [68], since they start meiotic prophase soon after completion of epigenetic reprogramming, thus requiring the timely expression of these genes. A slight hypermethylation at GRR gene promoters in the mouse E14.5 $Tet1^{-/-}$ PGCs, Hill and collaborators also demonstrate that TET1 stimulates transcription of GRR genes via a DNA demethylation-independent mechanism [80, 81]. In addition, TET1 may also enhance transcription through regulation of the levels of 5mC and 5hmC at non-promoter cis-active elements, such as enhancers [79]. TET1 might also have a critical role in the subsequent removal of aberrant residual and/or de novo DNA methylation [79]. This suggests that global reprogramming events require efficient protection from de novo DNA methylation to stabilize the newly acquired epigenetic state after the removal of 5mC. PGC epigenetic reprogramming entails complex erasure of epigenetic information and suggests that to enable gametogenesis, a timely and efficient activation of GRR genes is required [79].

Usually, a loss of DNA methylation in somatic cells induces an ectopic expression of retrotransposons, an anarchic proliferation, and apoptosis [82]. However, PGCs develop normally despite this

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hypomethylated state. The hypothesis is that the chromatin reorganization could enhance the genome stability and ensure proper chromosome alignment and segregation during mitosis, as well as global transcriptional quiescence during this developmental period [25, 58, 64, 71, 74, 83]. Studies in different models have shown that this process seem to be conserved in multiple species. Experiments with human PGCs have found that these DNA demethylation events follow the same patterns as the ones found in the rodents [72, 73, 84].

Histone marks. In a murine model, before the DNA methylation erasure, the pre-migratory PGCs start a process of reprogramming that erases epigenetic marks. One of the central epigenetic changes in pre-migratory and early migratory mouse PGCs is the loss of H3K9me2. This event is followed by an accumulation of H3K27me3 signal [74, 83, 85]. Eguizabal and collaborators showed in early human gonadal PGCs similar chromatin changes in the human early developing germ line [86]. An erasure of genomic imprints and dynamic changes in chromatin modifications are observed in the mouse PGCs after their entry in the genital ridge [74]. After week 9 of gestation, and similarly to porcine PGCs, human PGCs lose H3K27me3 [86].

Spermatogenesis & spermiogenesis

Male fertility relies on the production by the testis of large numbers of normal spermatozoa. This process is known as spermatogenesis, which can be divided in three major steps: (i) mitosis with the multiplication of the spermatogonia, (ii) meiosis to reduce the number of chromosomes from diploid to haploid cells, which starts with type B spermatogonia into the prophase of the first meiotic division. The cells are then called primary spermatocytes that then divide to form secondary spermatocytes, which will divide and undergo meiosis to form the round spermatids, (iii) and finally, the spermiogenesis, which refers to the successful maturation of round spermatids into spermatozoa [87]. All of these steps are central in the spermatogenic process, and any defect during the spermatogenesis can result in the reduction or absence of sperm production, or production of abnormal sperm (Figure 3).

Endocrine and paracrine regulation

FSH. Many studies in the rat have defined the stages at which testosterone and follicle stimulating hormone (FSH) act during spermatogenesis. The general consensus, until the mid-1990s, was that FSH was paramount for the initiation of spermatogenesis, but its role in the adult was to maintain a normal quantitative germ cell production [88, 89]. However, the study of transgenic mice lacking FSH or its receptor (FSHR) showed that the males were fertile but with a reduced germ cell number [90–94]. Closer observations of these KO mice revealed a reduction in spermatogonia, spermatocytes, and spermatids numbers, which suggests that FSH increases the number of spermatogonia and facilitates their entry into meiosis [90–94]. However, studies on *hpg*. SCARKO (hypogonadal mice lacking gonadotrophins and intratesticular androgen crossed with mice lacking androgen receptors specifically on the Sertoli cells) or *hpg*. ARKO mice (hypogonadal mice lacking gonadotrophins and intratesticular androgen crossed with mice lacking androgen receptors ubiquitously) have shown that FSH does not induce round spermatid formation [95–98].

While a lack of FSH action has been shown to impact spermatogenesis, it is difficult to determine the role of this hormone on maintaining this process. This is explained by the fact that FSH or its receptor are missing from the start of reproductive development. During normal adult spermatogenesis, apoptosis is a sporadic event, occurring mainly among spermatogonia. Lack of FSH, before sexual maturity and during the first wave of spermatogenesis, which is accompanied by an outburst of focal apoptosis among germ cells, increases the level of cells dying which may impact the adult spermatogenesis [99]. Other studies in rats have suggested that FSH-treatment acts to increase spermatogonia and spermatocyte numbers but displays a limited or incomplete effect on spermatogenesis [100, 101]. While these findings mostly agree with the data with the FSHRKO mice, there are significant differences. In these two studies, it was proposed that FSH could promote the completion of meiosis in rats, which was not observed in the transgenic mice models [101, 102]. The mechanism of action of FSH remains unclear even though FSH can act indirectly through Sertoli cells to increase spermatogonial differentiation/proliferation, but also modify rates of germ cell apoptosis [103–107]. A further role for FSH in the testis might be maintenance of Sertoli cell water balance as an accumulation of fluid was observed in FSHRKO mice cells [108]. As a result, this can alter cell morphology and interactions between germ cells and Sertoli cells, thus could reduce normal spermatogenic efficiency.

Androgen. Androgen plays an essential role in development and maintenance of spermatogenesis, which has been emphasized by a study demonstrating that the precocious expression of androgen receptors (ARs) in Sertoli cells leads to premature spermatogenic cell development [109]. The role of androgens has been clearly demonstrated in any animal model in which androgen levels are reduced, such as through hypophysectomy, GnRH-treatment (agonist or antagonist), ethanedimethane sulfonate treatment (EDS) (which ablates Leydig cells), or in gonadotrophin-deficient mice. In all of these cases, significant loss of pachytene spermatocytes and round spermatids, especially at stages VII and VIII of spermatogenesis, can be reversed by a treatment with testosterone [98, 110–117]. Moreover, in mice lacking functional androgen receptors (*tfm* or ARKO), there is a significant loss of spermatocytes which are also unable to complete meiosis and form round spermatids [118–121]. Androgen maintains indirectly through the Sertoli cells meiosis, which appears to ensure the survival of pachytene spermatocytes and enable diplotene spermatocytes to enter meiotic division [121]. However, the role of androgens in spermiogenesis and spermiation remains unclear in regard to its role on the germ cell niche. Most testis cell types express androgen receptors, except the germ cells. Several studies have concluded that androgen action in the testis is only mediated through somatic cell populations [107, 122, 123].

Estrogen. The physiological role of estrogens in the adult testis has yet to be completely understood. However, some studies suggest that estrogen action is required in the neonate to enable a normal spermatogenesis in adulthood. Estrogen has multiple indirect effects through endocrine regulation and through other tissues on the testis, which makes the study of its action even more difficult. In the adult *hpg* mouse, estrogens stimulate spermatogenesis by actually stimulating the FSH release from the pituitary [124, 125]. Interestingly, exogenous estrogens inhibit spermatogenesis in normal adult animals by inhibiting LH secretion and intratesticular testosterone levels [126]. In some species, the aromatase activity present in the testis can convert androgens to estrogens, such as the horse testes,

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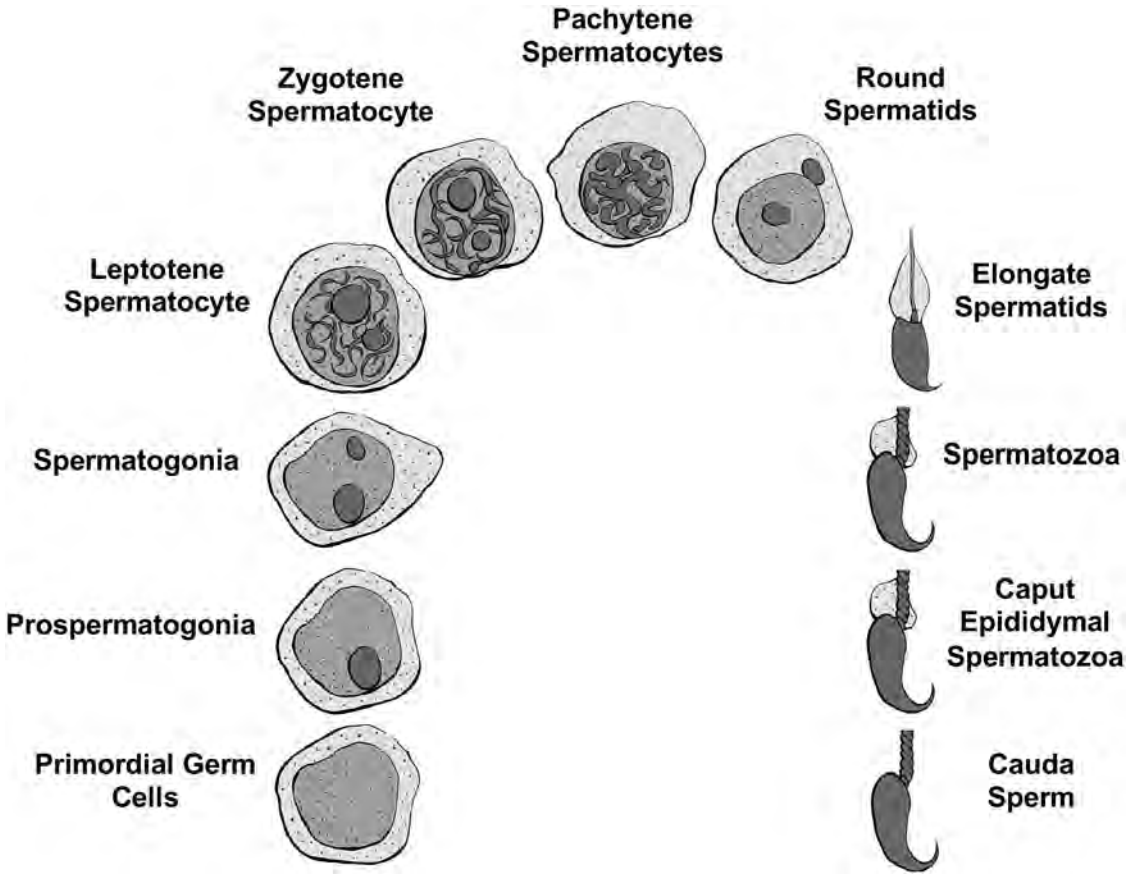

Figure 3. Gametogenesis and spermatogenic germ cell stages (modified from [162]).

which produce large concentrations of estrogens [127, 128]. Several cell types in the testis, including the germ cells, express nuclear estrogen receptors (ERα and ERβ), and the membrane cell receptor GPR30 [129, 130]. In ArKO mice (lacking aromatase), the males are initially fertile, but spermatogenesis degenerates and an arrest is observed in the early stages of spermiogenesis and multinucleated cells in the tubular lumen appear [131]. Moreover, during neonatal period, estrogen-dependent ERα signaling is required for a normal adult spermatogenesis and fertility [132].

Spermiogenesis seems to be clearly affected by estrogens. In fact, after irradiation damage to the testis, estrogens are able to stimulate spermatogonial differentiation [133, 134], which is not correlated to intratesticular testosterone suppression [135]. Numerous studies have shown that estrogens are involved in the early development of spermatogenesis and are able to affect spermatogenesis. However, their role in the normal adult spermatogenesis still needs to be determined.

Activin. The majority of testicular cell types produce activins and activin-related proteins [136]. Even though they can act as hormones, they also behave as growth factors in regard to spermatogenesis. Sertoli cells and germ cells express activin receptors [136]. Culture of stem spermatogonia cells, spermatogonia and spermatocytes has shown that these cells are sensitive to activin [137–139]. Follistatin (FST) and follistatin-like 3 (FSTL3) are two activin-binding proteins that can act as antagonists to activin activity. However, overexpression of FST does not reduce local activin levels but causes infertility without clear effects on FSH levels [140]. In KO mice for FSLT3, an increase in germ cell numbers was observed which was correlated to the increase in Sertoli cell numbers [141]. These different findings suggest that activins have probably a regulatory role in maintaining spermatogenesis and ensuring normal Sertoli cell development and activity.

Spermatogonial formation and renewal. In the mammalian testis, once the primordial germ cells migrate there during fetal development, they associate themselves with the mesenchymal cells, which will later give rise to the Sertoli cells. At this point, the sex cords are formed. The primordial germ cells then differentiate into prospermatogonia and remain centrally positioned in the cords surrounded by immature Sertoli cells. After a period of proliferation, postnatal in the rodent and prenatal in the human, the prospermatogonia migrate to the basement membrane of the sex cords to divide and form type A spermatogonia (Figure 3). Depending on the species, differences have been reported. In the human, A pale, A dark, and B spermatogonia types have been identified [142]. In the rodent testis, multiple type A spermatogonia, intermediate and type B spermatogonia have been reported and their appearance in the testis is temporally controlled. In the rat, around postnatal day 4 or 5, spermatogonial proliferation begins with type B spermatogonia identified at P6. After a series of mitotic divisions, whose mechanisms are only starting to be understood, during puberty the type B spermatogonia develop the capacity to develop into the preleptotene stage of the meiotic process.

In the testis, the migration of primordial germ cells depends on their surface expression of c-kit protein, which is the receptor for

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stem cell factor (SCF), produced by the immature Sertoli cells. Mutations of the c-kit receptor will result in failure of spermatogenesis due to the absence of germ cells from the testis [143]. An upregulation of SCF mRNA on E5 has been and is concurrent with the beginning of spermatogonial division [144], which indicates an interaction between c-kit and SCF to mediate and modulate spermatogonial proliferation. Other essential growth factors in the fetus testis include transferring growth factor alpha (TGFα) [145] and neurotrophic factors [146].

SCF and c-kit have also been shown to regulate the adult testis survival of spermatogonia and spermatocytes [147]. Rat tubules cultures with FSH have been shown to influence germ cell apoptosis, affecting both mitotic and meiotic cell populations [106, 148]. GDNF and CSF1 signaling are important for spermatogonial stem cell renewal in the stem cell niche [149, 150]. Survival and developmental progression of spermatogonia depend upon expression of several genes, including the transcription factor ID4 [151] and the RNA binding protein NANOS2 [152]. Different sub-populations of spermatogonial stem cells express different genes, depending on whether that population is undergoing self-renewal, differentiation and progression, or replenishment of earlier stem cell stages [153, 154].

Spermatogenic stage germ cell development and epigenetics. Many studies have shown that the dynamics of epigenetic modifications and their regulatory networks are essential for normal spermatogenesis. Any perturbations of these epigenetic modifications is likely to cause degrees of infertility and these perturbations could result in phenotypic defects in subsequent generations [3, 155–157]. Two studies have shown that a high fat or low protein in male mice can alter the metabolic gene expression in the offspring mediated by small noncoding RNAs (sncRNAs) derived from transition ncRNAs [158, 159]. Abnormal DNA methylation is associated with altered histone modifications, dysregulation of ncRNA, abnormal protamination, and all of these contribute to male infertility. In the prospermatogonia, prepachytene piRNAs are necessary for silencing mobile elements through guiding the *de novo* DNA methylations of transposable elements in order to guarantee genome stability [160]. In late spermatocytes and round spermatids, the pachytene piRNAs could silence the retrotransposon sequences through degrading the 3′UTR of retrotransposon mRNAs or recruiting the DNMT3L to the retrotransposon locus [161]. Most studies on the different spermatogenesis stages have focused on one type of cells. In the field of infertility, histone modifications are mostly studied in the mature sperm. Our lab investigated the developmental alterations in DNA methylation during gametogenesis from PGCs to sperm. Rat fetal PGCs, prospermatogonia, spermatogonia, meiotic pachytene spermatocytes, haploid round spermatids, caput spermatozoa and mature cauda sperm were isolated and purified. Differential DNA methylation regions between each developmental stage involved were compared. The study identified a dynamic cascade of epigenetic changes during development, the most dramatic happening during the early developmental stages, which suggests complex alterations to regulate genome biology and gene expression during gametogenesis [162].

Epididymal maturation and epigenetics. Although spermatogenesis is complete with the formation of the spermatozoa following spermatogenesis, additional maturation of the sperm occurs in the epididymis [163–166]. The spermatozoa released into the seminiferous tubules collect in the rete testes and pass through the efferent ducts into the head of the epididymis called the caput epididymis. The spermatozoa in the caput epididymis go through a further maturation as it passes through the caput to the corpus epididymis and finally, to the cauda epididymis. The caput epididymis spermatozoa do not have the capacity to have motility [167, 168]. During the transit through the epididymis, the epididymal epithelial cells produce proteins that are acquired and modify the maturation of the sperm to then in the cauda epididymis gain the capacity to become motile following ejaculation from the vas deferens where sperm are collected following epididymal maturation and stored. Therefore, the caput spermatozoa undergo a final stage of maturation during epididymal transit to the cauda epididymis to mature and gain the capacity to develop motility. The cauda epididymal sperm are then stored in the vas deferens. The molecular level maturation remains to be fully elucidated, but some aspects of epididymal maturation are known [169, 170].

Epigenetic alterations during epididymal maturation of the sperm largely remain to be elucidated [171]. Although the sperm nuclei is transcriptionally silent due to the compaction of DNA with protamines in testicular spermatogenesis, protein and epididymal components like ncRNA can be passed to the sperm and localized in the head of the sperm in the acrosome vesicle [171]. The localization of epigenetic components like ncRNA in the sperm nuclei remains to be established, but has been speculated in previous literature [172, 173]. Therefore, the role of epididymal ncRNA for sperm epididymal maturation requires further research, but is potentially an important epigenetic aspect of sperm maturation to consider [174].

Recent studies have investigated the epigenetic alterations between the caput epididymal spermatozoa and the mature cauda epididymal sperm. Environmentally induced (DDT and vinclozolin) epigenetic alterations in sperm have been shown to alter differential DNA methylation regions (DMRs) between the caput and cauda epididymal stage sperm [175, 176]. In addition, analysis of histone retention sites in the caput spermatozoa versus cauda sperm have shown differential histone retention regions (DHRs) [177]. Therefore, during epididymal maturation of sperm, there are DNA methylation and histone retention alterations that occur and are a further epigenetic developmental aspect of gamete development.

Oogenesis

Oogenesis involves the production of female gametes called eggs, and begins with the differentiation of primordial germ cells into oogonia at the period of sex determination [178]. In mammals, oogonia proliferate mitotically during fetal life to form a pool of primary oocytes that arrest in the prophase stage of the first meiotic division and stay in this state of meiotic arrest until the female reaches adulthood [179]. In the developing embryo, the nests of arrested oogonia are surrounded by somatic pre-granulosa cells [180]. Interaction and communication between oogonia and the surrounding somatic cells are vital for normal follicle and oocyte development [181, 182]. The oogonia nests subsequently break down, many oogonia undergo programmed cell death, and the pre-granulosa cells migrate to surround each remaining arrested oocyte in a process termed primordial follicle assembly [183, 184] (Figure 4). A primordial follicle is composed of a single oocyte surrounded by a single layer of flattened pre-granulosa cells. Oocytes are maintained in primordial follicles until sexual maturity, at which point follicles begin to be recruited out of the pool of primordial follicles and undergo primordial to primary follicle transition [185, 186].

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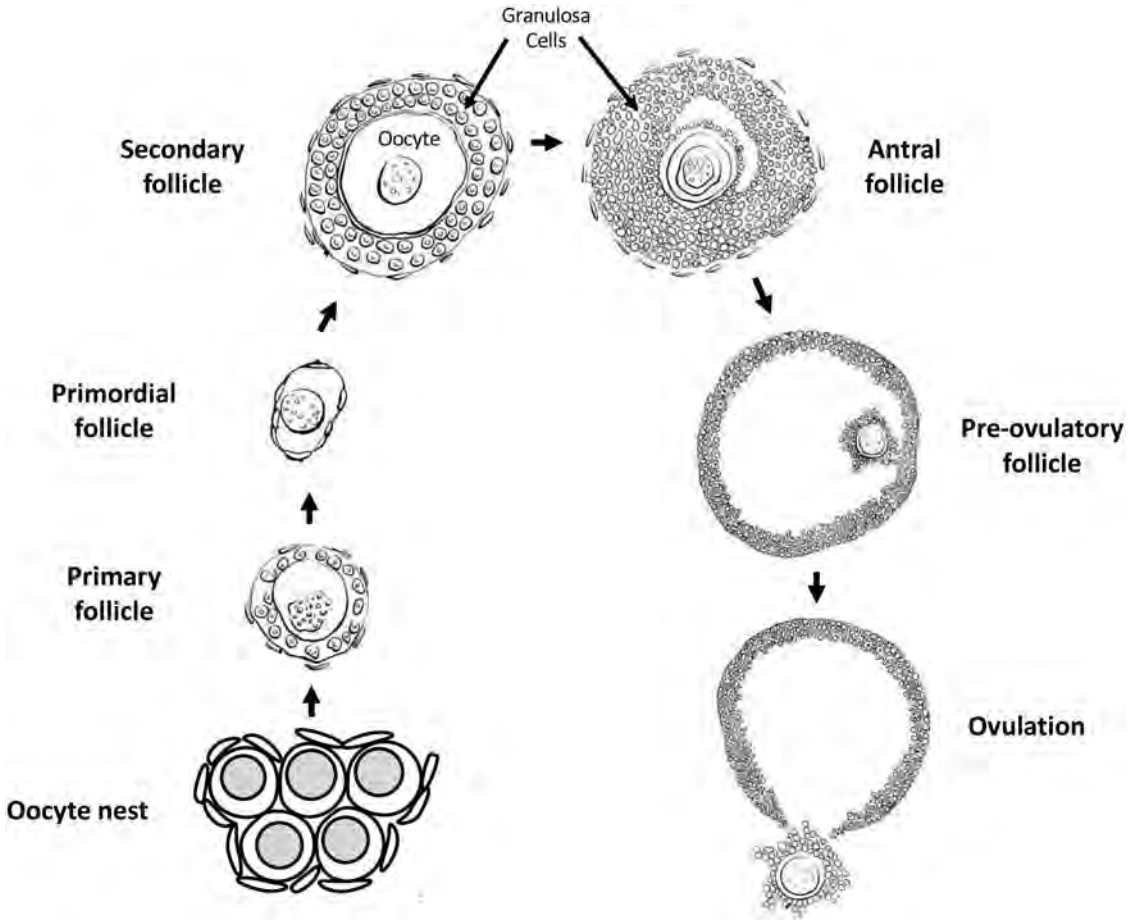

Figure 4. Oogenesis and ovarian follicle stages.

Once a primordial follicle undergoes transition and begins developing, the surrounding flattened pre-granulosa cells become cuboidal and begin proliferating, themselves surrounded by the ovarian stromal cells destined to become theca cells. The follicle is now termed a primary follicle [180]. Subsequent growth and development of the follicle into secondary and pre-antral follicle stages involves continued proliferation of the granulosa cells to form multiple layers, and the initial development of a theca cell layer around the granulosa cells. Follicles with multiple layers of granulosa cells gain sensitivity to follicle stimulating hormone (FSH) secreted by the pituitary, and thus are regulated to grow and develop in cyclic waves in coordination with the estrous cycle [187]. As the granulosa and theca cell layers proliferate a fluid-filled space or antrum forms in the follicle, eventually dividing the granulosa cells into cumulus granulosa surrounding the oocyte and mural granulosa around the inside periphery of the follicle [188]. Extensive cell–cell communication and growth factor signaling between the oocyte and somatic cells occurs at all stages of oogenesis [181, 182]. Most developing follicles do not reach the stage at which ovulation occurs. Rather, follicles at several stages undergo atresia and regress [189–191], (Figure 4).

A luteinizing hormone (LH) surge from the pituitary induces ovulation in late-stage pre-ovulatory follicles, as well as promoting the resumption of meiosis in the oocyte [192]. Meiosis progresses in ovulated oocytes through the production of the first polar body, and then arrests again in metaphase two of the second meiotic division until the time of fertilization. If fertilization occurs, then meiosis again resumes and progresses to completion with the production of the second polar body and the formation of the female pronucleus. Syngamy is the fusion of the male and female pronuclei in the newly formed zygote [193, 194]. Subsequently, the zygotic genome is activated in a carefully controlled manner to allow expression of needed genes in the newly formed individual, while suppressing expression of undesirable genes such as retrotransposons [195, 196].

Epigenetics during oogenesis and in oocytes

Information about the normal epigenetic changes that occur during oogenesis is limited. This is in part due to the difficulty of evaluating developing oocytes in ovaries, and the relatively small number of oocytes available for isolation and study compared to what can be done with male germ cells. Nonetheless, some knowledge of normal epigenomic development in female germ cells has been determined.

Primordial follicle assembly, primordial to primary follicle transition, and many subsequent stages of oogenesis have been shown to be regulated by small non-coding RNA expression, as detailed later in the *non-coding RNAs* section of *Epigenetic Programming During Gametogenesis* [197–200], (Figure 4). DNA methylation occurs at sites that are differentially imprinted between male and female gametes so that imprinted genes can be mono-allelically expressed in offspring. DNA methylation is gained gradually on imprinted genes in oocytes in developing follicles after the primary follicle stage, continuing through antral follicle stages of development [201,

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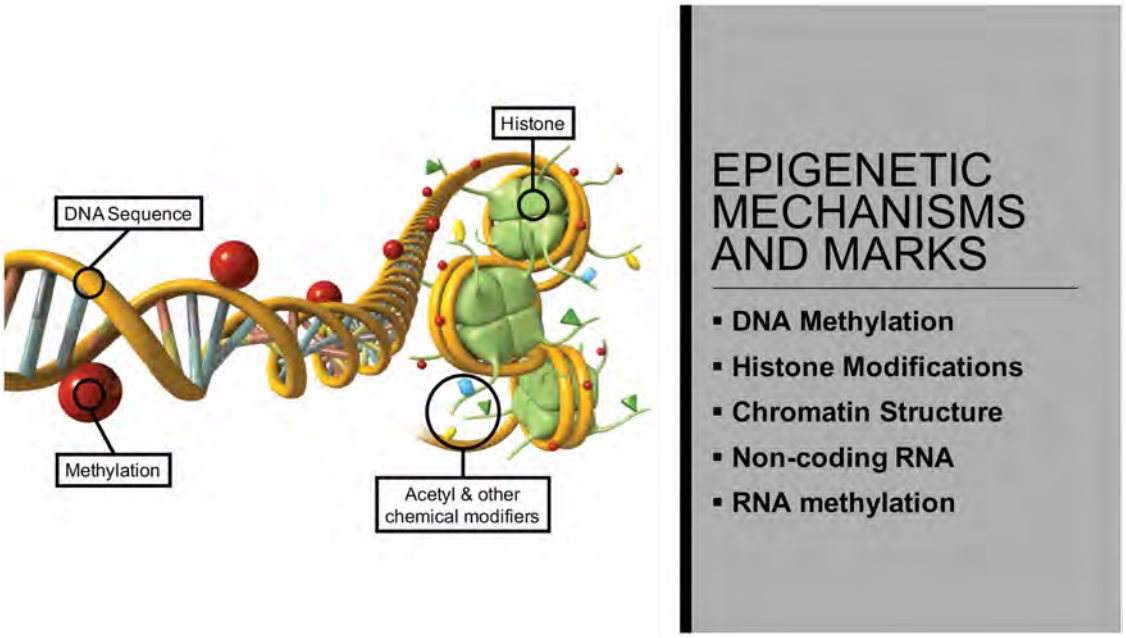

Figure 5. Epigenetic mechanisms and processes (marks) (modified from [330]).

202]. In the oocytes of antral stage follicles and later, H3K4Me3 histone methylation increases. This is important for normal function in mature oocytes, and is involved with establishing the DNA methylation pattern in mature oocytes [203–206].

Using *in vitro* xenogeneic reconstituted ovaries (xrOvaries) with mouse embryonic ovarian somatic cells, Yamashiro and collaborators studied if human primordial germ cell–like cells (hPGCLC) can undergo further development [207]. They observed around 80% of genome-wide 5mC levels in human-induced pluripotent stem cells (hiPSCs) and incipient mesoderm-like cells (iMeLCs) which decreased progressively to around 20% in hPGCLC-derived cells in culture day 77 cells, then dropped at around 13% in culture day 120 cells. Based on their data, the demethylation occurred throughout the genome. Moreover, the 5mC distribution profiles of the culture days 77 to 120 cells were comparable to those observed in the oogonia and gonocytes at weeks 7 to 10. However, they were different to those seen in the blastocysts [208] and naïve human embryonic stem cells (hESCs) [207, 209]. This study demonstrated that hPGCLC-derived cells demethylate their 5mCs similarly to that of oogonia and gonocytes but not early embryonic cells and their putative in vitro counterparts [207].

Epigenetic programming during gametogenesis

Epigenetics

Epigenetics refers to 'the molecular factors and processes around the DNA that regulate genome activity independent of DNA sequence, and that are mitotically stable' [3]. These molecular processes include DNA methylation, chromatin structure, histone modifications and retention, non-coding RNAs, and RNA methylation (Figure 5). The epigenome is the complex integration of epigenetic modifications. The first epigenome analysis mapped histone acetylation and methylation in yeast [210]. These epigenetic processes and factors are central for an organism to respond to its environment with changes in gene expression. Moreover, epigenetic mechanisms are required for a stem cell type to develop into a differentiated cell type, which make them an integral part of normal biology [3, 211, 212].

DNA methylation and histone modifications

De novo DNA methylation in males restarts in prospermatogonia at E14.5 in mice and is fully established at birth [213, 214]. In both female and male germlines, the factors responsible for mediating DNA methylation DNMT3A or 3B and DNMT3L at imprinted loci were identified [215–217]. In the gametes, the sequence identity and the characteristics of imprinted regions are now well characterized; however, the mechanisms targeting the *de novo* methyltransferases to imprinted regions remain to be further investigated [218]. In oocytes, the establishment of DNA methylation at imprinted or retrotransposon-rich sites occurs gradually in growing follicles subsequent to the primary follicle stage (reviewed in [201, 202]).

During meiosis of the gametogenesis process, germ cells stop their progress at the prophase stage to allow parental genomes to exchange genetic information through meiosis recombination [219]. At this stage, chromosomes pair in a homologous manner, and large pieces of the chromosomes can be exchanged through crossover events [219, 220]. These crossover events are necessary to maintain euploidy in gametes. An absence of crossover events has been linked to infertility and aneuploidy in the offspring [221, 222]. These meiotic crossover events happen at genomic hotspots and are enriched in regions outside of promoters that bear histone H3K4me3 peaks and established by Prdm9 [223–228]. Mutations in enzymes involved in histone posttranslational modifications observed in meiosis have been shown to have an impact (decrease or increase) on the DNA double-strand break activity, which suggests a role for histone modifications in the initiation and/or repair activity [229–232]. In oocytes, H3K4Me3 histone methylation increases from the antral follicle stage onward, and is important for meiotic recombination, oocyte maturation, oocyte transcriptional activity, and for the establishment of a normal DNA methylation pattern in mature oocytes [203–206, 233, 234].

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Chromatin structure & histones

The chromatin reorganization during meiosis is largely transient. The most extensive modifications in chromatin state, structure, or composition occurs after male and female meiosis [220]. In the sperm, the vast majority of histones are replaced with sperm-specific nuclear proteins called protamines [235]. This process is facilitated by different steps: hyperacetylation of histones in round spermatids believed to weaken the interactions between histones and DNA, this will enable the eviction and replacement of histones by testes-specific histone variants, then by transition proteins to end with protamines [236–238]. Because of their endonuclease-inaccessible toroid structure, protamines manage to package the sperm DNA into a tenfold more compact structure than the heterochromatin found in somatic nuclei [239, 240]. The retained histones in the sperm were believed to be remnants of incomplete histone-to-protamine replacement, but recent studies have demonstrated that these retained histones are present at key developmental gene promoters/enhancers in mature sperm. These retained histones bear both active or repressive histone modification [241, 242].

This programmatic retention and evolutionary conservation of histone localization suggests that epigenetic information can be passed through the paternal lineage. Moreover, alteration in histone levels, or chromatin regulators involved in spermatogenesis leads to developmental defects, which can be passed on to the subsequent generation [243, 244]. Altogether, these studies imply that retained histones serve as molecular carriers of epigenetic memory; however, the mechanisms are yet to be elucidated.

Non-coding RNAs

DNA is not the only means to transmit the information between generations. Non-coding RNAs (ncRNAs) are regulatory elements of gene expression and chromatin structure [245]. The differential susceptibility to these non-coding RNAs contributes to tissue-specific gene expression. Early on, ncRNAs are important in the germline development, but they are also crucial players in posttranscriptional gene control during spermatogenesis and oogenesis. Different classes of ncRNAs exist, but this section will focus on microRNAs (miRNAs), Piwi interacting RNAs (piRNAs), and long non-coding RNAs (lncRNAs) and their role in the PGCs and the gametes.

miRNAs

During the PGC specification, some miRNAs are selectively expressed such as miR-10b, −18a, −93, −106b, −126-3p, −127, −181a, −181b, and − 301. All of them have important functions in these cells such as differentiation, migration, and apoptosis in PGCs in mice [246]. For instance, Medeiros and collaborators have shown that in mice a deficiency in miR-290-295 cluster result in an abnormal germ cell with defect in the PGC migration [247]. In the female, an upregulation of miR-29b has been shown to induce PGC development by targeting DNA methyltransferases Dnmt3a and Dnmt3b [248]. In the zebrafish, miR-202-5p has been identified as a potential germ plasma-specific biomarker due to its potential role in the germ cell development [249]. Other microRNAs have been linked to PGC migration in the zebrafish as well, such as miR-430, which regulates *sdf1a* and *cxcr7* mRNAs key transcripts regulating migration [250].

In the early stages of spermatogenesis, different miRNAs have been described in mammals as being crucial for germ cell self-renewal and differentiation. miR-34c has been identified as promoting mouse spermatogonial stem cell (SSCs) differentiation by targeting Nanos2 [251]. Moreover, this miRNA has another role in the later stage of spermatogenesis where miR-34c is involved in apoptotic events of spermatocytes and round spermatids [252], and also in the NOTCH signaling, which is important in the control of germ cell differentiation [253]. A list of miRNAs involved in cell cycle regulation have been identified such as miR-293, 291a-5p, 290-5p and 294 [254]. Other miRNAs are involved in later stages of spermatogenesis. The Let-7 miR family is involved in the mouse spermatogonial differentiation, especially in the maturation of undifferentiated spermatogonia to A1 spermatogonia by suppressing Lin28 [255]. In contrast, some miRNAs such as miR-146 play a crucial role in keeping spermatogonia in an undifferentiated state in the mouse [256]. Other miRNAs play a role in the regulation of meiotic and postmeiotic events in the later stages of spermatogenesis such as the miR-449 cluster. During murine spermatogenesis, the upregulation of miR-449 cluster is crucial for the initiation of meiosis [257]. These miRNAs by targeting BCL2 and AFT1 are involved in germ cell apoptosis [258].

piRNAs

Another class of sncRNAs, piRNAs have been discovered in the germline. Their role is to safeguard the germline genome from retrotransposons and protect the genomic stability [259, 260]. These piRNAs are believed to be involved in pathway components of DNA methylation remodeling during early PGC specification in mammals [209]. Moreover, a loss of Piwi function in mice or zebrafish results in a decrease of germ cells by apoptosis, this underlying its role in germ cell maintenance [261].

lncRNAs

The role of long non-coding RNAs in PGC specification has not been described. Some researchers suggest their possible roles in controlling transcription factors such as BLIMP1/PRDM1 or DAZL [262, 263]. In fact, more than 300 binding sites of BLIMP1/PRDM1 in the murine PGCs are associated with non-coding genes whose functions in PGCs specification are still unknown [23, 262]. The lncRNA-Tcam1 and lncRNA-HSVIII have a crucial role in pachytene spermatocytes, which implies their potential participation in the transcriptional regulation of spermatocyte-specific gene expression [264]. LncRNAs have been linked to functions related to post-transcriptional control during spermatogenesis, such as tubulin cofactor A (TBCA), which has the ability to interact with tubulin during the microtubule rearrangement process [265]. However, most studies have been conducted in rats, so not much is known about lncRNAs in the human. In human spermatogenesis, male infertility has been associated with NLC1-C through the control of miRNA expression via RNA-binding proteins [266].

ncRNAs in oocytes

Most of the studies have focused on miRNAs in oogenesis and ovary function using conditional KO mice models to evaluate their involvement in the ovary. By using this approach, a clear role has been outlined for miRNAs in folliculogenesis, oocyte maturation, and ovulation. Other miRNAs are also involved in the assembly of primordial follicles, the transition from primordial to primary follicles, follicular growth, oocyte maturation, ovulation, and the formation of the *corpus luteum* in mammals [198, 199, 267–269]. After a conditional knockout of Dicer1 from follicular granulosa cells in mammals, abnormal oocyte maturation, disrupted follicular

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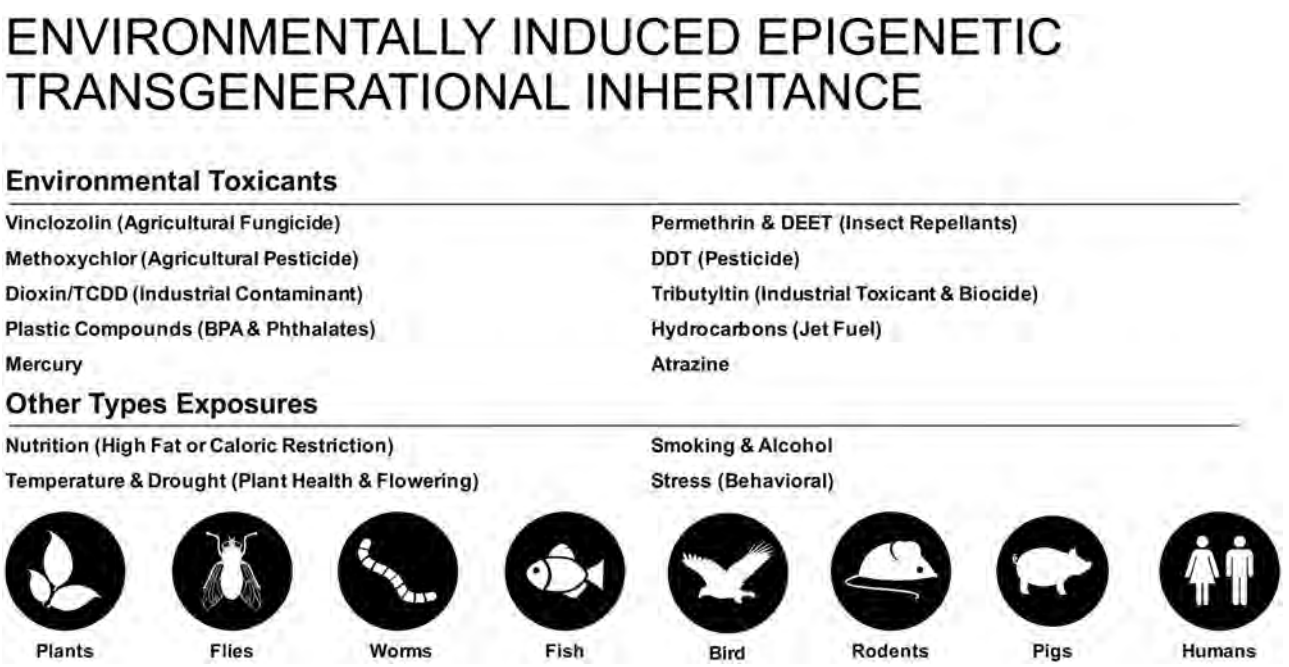

Figure 6. Environmentally induced epigenetic transgenerational inheritance. Various exposures and species investigated (modified from [156]).

development and ovulation, increased follicular atresia, and infertility were reported [270–272]. Other studies have demonstrated miRNAs involvement in granulosa cells proliferation, survival, terminal differentiation, steroidogenesis, and cumulus expansion [200, 273–281]. The overexpression of miR-143 in murine 15.5 dpc ovaries has been shown to repress the formation of primordial follicles by stopping the proliferation of pre-granulosa cells. An increased number of primordial follicles were observed in transfected 18.5 dpc ovaries with miR-376a (reviewed by Grossman and Shalgi [197]).

Environmental toxicant exposures resulting in epigenetic changes in gametes

In addition to epigenetic changes being a part of the normal developmental process for gametes, it is also possible that exposure to environmental factors will induce abnormal epigenetic changes to the germ cell epigenome [282, 283]. Such changes may be heritable and affect the phenotype of subsequent generations [156, 284]. Exposure of male mice to the endocrine disruptor bisphenol A (BPA) induces changes to DNA methylation in the fetal germ cells of their developing offspring [285]. Primordial germ cells are also induced to alter DNA methylation in response to hypoglycemic conditions in the uterus [286] or from exposure to the agricultural fungicide vinclozolin [287]. Exposure to a wide variety of environmental factors can lead to DNA methylation changes in spermatozoa and mature sperm [288]. In rodents, direct exposure to arsenic [289], the fungal toxin zearalenone [290], the plastics compounds bisphenol A (i.e. BPA) [291] and phthalates [292], the agricultural fungicide vinclozolin [293], the pesticide dichlorodiphenyltrichloroethane (DDT) [294], and the herbicides glyphosate [295] and atrazine [296], all induce sperm DNA methylation changes. In humans, studies have shown that environmental factors such as exposure to phthalates [297], alcohol [298], flame retardants [299, 300], chemotherapy treatment [301], obesity [302], and exercise [303] are correlated to sperm DNA methylation changes (Figure 6). In one fish species, exposure to BPA resulted in changes to oocyte DNA methylation in the next generation [304], (Table 1).

Epigenetic changes to histones in germ cells can occur after exposure to environmental factors. For example, zebrafish exposed to BPA showed decreased sperm histone acetylation, as well as impaired primordial germ cell migration, although these findings were not associated with decreased fertility [305]. BPA exposure in a minnow species resulted in changes in oocyte histone methylation in the offspring [304]. Pubertal exposure of mice to the fungicides carbendazim and chlorothalonil caused changes in H3K9me3 levels in sperm [306]. Pubertal exposure to the fungal toxin zearalenone altered mouse histone H3K27 methylation [290]. In mice, exposure to the pesticide chlordecone resulted in altered levels of H3K4Me3 in developing testes [307]. Exposure to chlordecone in mice also resulted in changes in H3K4me3 and H4ac in mature oocytes [308]. Even exposure to chronic restraint stress can alter histone acetylation, methylation and phosphorylation in germinal vesicle-stage oocytes [309].

Another way in which environmental factors can alter histones in sperm is to affect which histones are retained as male germ cells develop. As male germ cells undergo spermiogenesis, most of the histones associated with the DNA are replaced by protamines [236, 310]. Protamines help condense and package DNA into the small sperm head. However, some histones are retained, and they are often located near developmental regulatory genes that are expressed early in embryonic development [311]. Exposure to environmental toxicants has been shown to alter retention of histones in sperm [312]. Men exposed to either cigarette smoke [313] or the smoke of surrounding fires [314] have been shown to have an altered ratio of histones to protamines in sperm. *In utero* exposure to caloric restriction in mice has also been shown to alter histone retention in sperm [315]. In transgenerational studies in rats, it was found that exposure of gestating female F0 generation rats to either DDT or vinclozolin resulted in changes in histone retention in the subsequent transgenerational F3 generation, but interestingly not in

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Table 1. Environmental exposures resulting in epigenetic changes in gametes.

Environmental exposure	Epigenetic change	Cell type	Reference
BPA	DNA methylation	Fetal germ cells	Zhang et al. (2012) [285]
Uterine hypoglycemia	DNA methylation	PGCs	Ren et al. (2018) [286]
Arsenic	DNA methylation	Sperm	Nohara et al. (2019) [289]
Zearalenone	DNA methylation	Sperm	Gao et al. (2019) [290]
BPA	DNA methylation	Sperm	Rahman et al. (2020) [291]
Phthalates	DNA methylation	Sperm	Prados et al. (2015) [292]
Vinclozolin	DNA methylation	Sperm	Beck et al. (2017) [293]
DDT	DNA methylation	Sperm	Skinner et al. (2018) [294]
Glyphosate	DNA methylation	Sperm	Kubsad et al. (2019) [295]
Atrazine	DNA methylation	Sperm	McBirney et al. (2017) [296]
BPA	DNA methylation	Fish oocyte	Zhu et al. (2020) [304]
Phthalates	DNA methylation	Human sperm	Wu et al. (2017) [297]
Alcohol	DNA methylation	Human sperm	Ouko et al. (2009) [298]
Flame retardants	DNA methylation	Human sperm	Soubry et al. (2017), Greeson et al. (2020) [299, 300]
Chemotherapy	DNA methylation	Human sperm	Shnorhavorian et al. (2017) [301]
Obesity	DNA methylation	Human sperm	Soubry et al. (2016) [302]
Exercise	DNA methylation	Human sperm	Denham et al. (2015) [303]
BPA	Histone acetylation	Fish sperm	Lombo et al. (2019) [305]
BPA	histone methylation	Fish oocyte	Zhu et al. (2020) [304]
Carbendazim and chlorothalonil	Histone H3K9me3	Sperm	Li et al. (2018) [306]
Zearalenone	Histone H3K27 methylation	Sperm	Gao et al. (2019) [290]
Chlordecone	Histone H3K4Me3	Developing testes	Gely-Pernot et al. (2018) [307]
Chlordecone	Histone H3K4Me3	Oocytes	Legoff et al. (2019) [308]
Restraint stress	Histone acetylation, methylation, phosphorylation	Oocytes	Wu et al. (2015) [309]
Cigarette smoke	Histone retention	Human sperm	Hamad et al. (2014) [313]
Smoke	Histone retention	Human sperm	Lettieri et al. (2020) [314]
Caloric restriction in utero	Histone retention and DNA methylation	Sperm	Radford et al. (2014) [315]
DDT	DNA methylation, non-coding RNA expression, and histone retention	Sperm	Skinner et al. (2018) [294]
Vinclozolin	DNA methylation, non-coding RNA expression, and histone retention	Sperm	Ben Maamar et al. (2018) [316]
Vinclozolin	miRNA	PGCs	Brieno-Enriquez et al. (2015) [317]
Early life trauma	miRNA and lncRNA	Sperm	Dickson et al. (2018), Gapp et al. (2014, 2020) [318–320]
Early life stress	miRNA	Human sperm	Dickson et al. (2018) [318]
Smoking	miRNA	Human sperm	Marczylo et al. (2012) [321]
Obesity	miRNA	Human sperm	Lopez et al. (2018), Donkin et al. (2015) [322, 323]
Bariatric surgery	miRNA	Human sperm	Donkin et al. (2015) [323]

the intervening F1 and F2 generations [294, 312, 316]. However, F1, F2 and F3 exposure-lineage generations all showed changes in DNA methylation and non-coding RNA expression (Table 1).

The expression of non-coding RNA (ncRNA) in germ cells is another epigenetic factor that can be responsive to environmental factors. Mice exposed to vinclozolin *in utero* exhibited changes in micro-RNAs in their primordial germ cells [317]. Rats exposed to either vinclozolin or DDT *in utero* have been shown to have altered levels of piwi-interacting RNAs (piRNAs) and small tRNA fragments in sperm upon reaching adulthood [294, 316]. Traumatic stress can also be an environmental factor that causes epigenetic changes in sperm. In mouse models of early life trauma, changes in miRNAs and long non-coding RNAs were seen in sperm from exposed males [318–320]. Interestingly, the behavioral and metabolic alterations seen in the resulting offspring were recapitulated by injection of sperm RNAs from traumatized males into fertilized wild-type oocytes [319]. In humans, changes in miRNA expression in sperm have been seen after exposure to early life stress [318], smoking [321], obesity [322, 323], and bariatric surgery [323] (Table 1).

Epigenetic transgenerational inheritance

Exposure to environmental factors, such as toxicants, can induce epigenetic changes in germ cells that can affect the subsequent generations. These epimutation changes are brought to the next generation at fertilization and have the possibility of altering gene expression and phenotype in the developing embryo. Since the germ cell epimutations can affect the earliest stem cells formed in the

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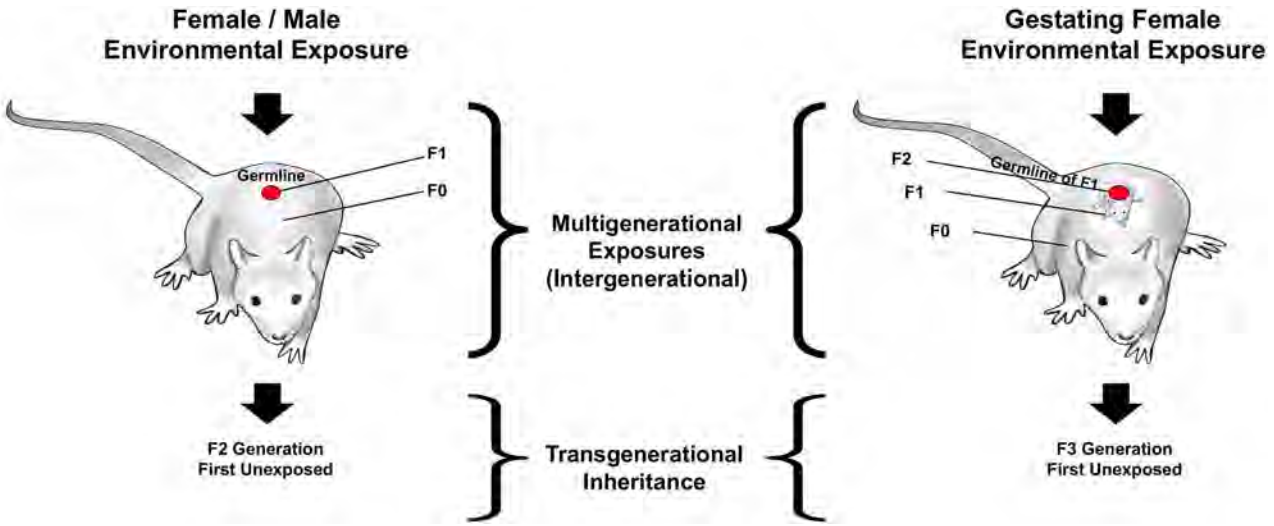

Figure 7. Environmentally induced transgenerational epigenetic inheritance. Schematic of multigenerational versus transgenerational environmental exposures (modified from [324]).

embryo, then any subsequent cell type in the embryo and adult animal may have epimutations and changes in gene expression that could affect their phenotype [156].

Epigenetic transgenerational inheritance is defined as germline-mediated inheritance of epigenetic information between generations in the absence of continued direct environmental influences that leads to phenotypic variation [3]. When a male or a non-pregnant female is exposed to an environmental factor that can induce epigenetic change, then epimutations can arise in that individual (the F0 generation), and that individual's germ cells. The germ cells that contribute to forming the next F1 generation were directly exposed to the environmental factor, so epigenetic and phenotypic changes seen in the F1 generation are an example of direct multigenerational exposure, but not transgenerational inheritance [324], (Figure 7). If these F1 generation animals pass on epigenetic and phenotypic changes to the unexposed F2 generation, then this is an example of epigenetic transgenerational inheritance. Similarly, if a pregnant female is exposed to an environmental factor such as a toxicant, the developing F1 generation embryo is directly exposed, and the germ cells in that embryo that go on to form the F2 generation are also directly exposed. Epigenetic and phenotypic changes would have to be seen in subsequent F3 generation or later generations for this to be an example of epigenetic transgenerational inheritance (Figure 7, Table 2) [156, 157, 324].

Epimutations have been reported in transgenerational generation gametes after ancestral exposure to a wide variety of environmental stressors including toxicants [156] and psychological stress [325]. This phenomenon has been observed in several organisms including fish [326] and rodents [157] (reviewed in [156]) (Table 2). Epimutations have been reported in transgenerational F3 generation rat sperm after ancestral exposure to several toxicants [156]. One question that occurs is at what stage of germ cell development do these sperm epimutations arise, or are they continuously present? Two recent studies have addressed this question. The first examined differences in DNA methylation compared to controls after ancestral exposure to DDT [176]. The number of differential DNA methylation regions (DMRs) was determined for the spermatogenic stages of primordial germ cells, prospermatogonia, spermatogonia, pachytene spermatocytes, round spermatids, caput epididymal spermatozoa, and cauda epididymal sperm. It was found that, of the 265 DMRs present in cauda epididymal sperm, 26% arose in the prospermatogonia stage, 25% in spermatogonia, 18% in pachytene spermatocytes, 5% in round spermatids, 12% in caput epididymal spermatozoa, and 14% only arose in cauda epididymal sperm (Figure 3). Similar observations were made with ancestral vinclozolin exposure [327]. This shows that transgenerational changes in DNA methylation arise throughout gametogenesis.

Similarly, another study examined transgenerational differences in histone retention in sperm from rats ancestrally exposed to DDT or vinclozolin [177]. In mature sperm nuclei, most histone proteins are replaced by protamines to facilitate packaging the DNA into the small sperm head [328]. However, some histones are retained, sometimes near genes that are important to early embryo development [243, 329]. In this study [177], the sites of differential histone retention (DHR) were determined for the spermatogenic stages of round spermatids, caput epididymal spermatozoa, and cauda epididymal sperm. It was found that, of those DHRs that were present in cauda epididymal sperm, about 50% were present in round spermatids, very few arose in caput epididymal spermatozoa, and 40–50% arose at the cauda epididymal sperm stage. Again, this shows that an environmentally induced cascade of histone retention changes occurs during different stages of spermatogenic development.

Recently, two studies investigated the integration of DMR, ncRNA, and DHR by overlapping the different genomic sites between the different epimutations. Rats were ancestrally exposed to DDT or vinclozolin and the F1, F2, and F3 generations sperm epimutations examined. The chromosomal locations of the DMR, ncRNA, and DHR epimutations were distinct with few overlapping, but present in the same regions with similar genomic features such as CpG deserts. Comparing the F1, F2, and F3 generations provided insights into the integration of the different epimutations and the direct exposure versus transgenerational impacts of exposures. Interestingly, the effects observed in the F3 generation were different than those observed in the direct exposure F1 and F2 generations. Direct exposure impacted the ncRNA in the F1 generation, but to a much lesser extent in the F2 and F3 generations.

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Table 2. Environmental exposures promoting epigenetic transgenerational inheritance.

Environmental exposure	Epigenetic change	Cell type	Reference
Several toxicants (review)	DNA methylation	Sperm	Nilsson et al. (2018) [156]
DDT	DNA methylation	Sperm	Ben Maamar et al. (2019) [176]
DDT	DNA methylation, non-coding RNA, and histone retention	Sperm	Skinner et al. (2018) [294]
Vinclozolin	DNA methylation, non-coding RNA expression, and histone retention	Sperm	Ben Maamar et al. (2018) [316]
Vinclozolin	DNA methylation	Sperm	Anway et al. (2005) [157]
DDT or vinclozolin	Histone retention	Sperm	Ben Maamar et al. (2020) [177]
Glyphosate	DNA methylation	Sperm	Kubsad et al. (2019) [295]
Atrazine	DNA methylation	Sperm	McBirney et al. (2017) [296]
Methoxychlor	DNA methylation	Sperm	Manikkam et al. (2014) [331]
Glyphosate	DNA methylation and histone retention	Sperm	Ben Maamar et al. (2020) [332]
Dioxin	DNA methylation	Sperm	Manikkam et al. (2012) [333]
BPA	DNA methylation	Sperm	Rahman et al. (2020) [291]
Phthalates	DNA methylation	Sperm	Prados et al. (2015) [292]
Jet fuel	DNA methylation	Sperm	Manikkam et al. (2012) [334]
Vinclozolin	tRNA halves	Sperm	Schuster et al. (2016) [335]
Methylmercury	DNA methylation	Fish sperm	Carvan et al. (2017) [326]
Nutrition change	Gene promoter methylation	Pig liver	Braunschweig et al. (2012) [336]
Famine	DNA methylation	Human blood cells	Jiang et al. (2020) [337]
Genetic manipulation	Histone modifications	*Drosophila* embryos	Xia et al. (2016) [338]
Genetic manipulation	Histone modifications	*Caenorhabditis elegans* larvae	Kelly et al. (2014) [339]
Vinclozolin	tRNA halves	Sperm	Schuster et al. (2016) [335]

Table 3. Future research in gametogenesis.

1)	Examine all stages PGCs to gametes for epigenetic and genetic transitions.
2)	Examine and investigate all the epigenetic processes (DNA methylation, histones, ncRNA, and chromatin structure) for the epigenetic regulation of gametogenesis.
3)	Use systems biology and genome-wide analyses to integrate the epigenetics and genetics of the gametogenesis process.
4)	Incorporate environmentally induced epigenetic transgenerational inheritance into our research and understanding of generational impacts of altering gamete epigenetics.

DMR were predominant in the F1, F2, and F3 generations, whereas the DHRs were observed primarily in the F3 generation. Observations suggest a potential role for ncRNA-directed DNA methylation and DNA methylation-directed histone retention in the environmentally induced epigenetic transgenerational inheritance of altered gametogenesis [294, 316].

Conclusions and future research

Normal fertilization and early embryonic development require the integration of genetic and epigenetic processes in the gametes. Few developmental or physiological processes will not require the integration of these two distinct molecular mechanisms. Understanding the underlying processes and mechanisms *in vivo* will help us better understand the gametogenesis and formation of the sperm and egg, as well as pathologies such as idiopathic infertility. The past few decades have advanced our knowledge of primordial germ cell and gamete development. This has provided insights into the molecular mechanism involved in related disorders, such as infertility and birth defects. These findings are also promising in the field of reproductive therapies.

Although a genetic focus can identify the genes involved in a developmental process such as gametogenesis, an understanding of the epigenetics is required to clarify how the gene expression is controlled and environmental factors regulate the developmental process. The DNA methylation, histone modifications, ncRNA, and chromatin structure are the epigenetic mechanisms required to regulate the gene expression. Therefore, no development or biological process can be understood without the integration of epigenetics and genetics. This is the case for normal developmental processes like spermatogenesis and oogenesis, as well as abnormal processes involved in pathology. The ability of environmental factors such as nutrition, toxicants, or stress to impact developmental processes like gametogenesis involves alterations in the epigenetics to then impact the basic genetics and gene expression. Since the gametogenesis process produces the gametes, sperm and eggs, alterations in gametogenesis impact the future generations physiology, phenotype, and gametes. This non-genetic form of inheritance termed epigenetic transgenerational inheritance requires manipulation during gametogenesis to obtain epigenetically modified sperm and eggs. Therefore, the current review provides the basic information on the gametogenesis process, epigenetic regulation of the developmental process, and impacts on epigenetic transgenerational

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inheritance. As the information on epigenetic regulation of gametogenesis is a new area of research, future research is needed to expand our understanding of the integration of epigenetics and genetics in gametogenesis.

The future research suggested includes the following, Table 3. A need to examine all the gametogenesis stages from PGCs to gametes to understand the integration and transition between the different stages of development. A need to integrate all the epigenetic processes and gene expression for a more complete understanding of the epigenetic regulation of gametogenesis. Emphasis on genome-wide analysis and system biology approaches in the study of gametogenesis to fully understand the developmental process and associated pathology. A need for a generational context, to incorporate the environmentally induced epigenetic transgenerational inheritance of gametogenesis alterations into our understanding of gamete development. Without these more complete systems biology approaches, the progress of research and impacts will be diminished.

Supplementary material

Supplementary material is available at *BIOLRE* online.

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Abbreviations

Primordial germ cells (PGCs)
Bone morphogenetic protein (BMP).
Knock-out (KO).
Stromal cell-derived factor 1 (SDF-1).
Chemokine receptor type 4 (CXCR4).
5-methylcytosine (5mC).
Germline reprogramming responsive (GRR).
Ten eleven translocation (TET).
Follicle stimulating hormone (FSH).
Androgen receptors (ARs).
Ethanedimethane sulfonate treatment (EDS).
Follistatin (FST).
Follistatin-like 3 (FSTL3).
Stem cell factor (SCF).
Luteinizing hormone (LH).
Non-coding RNAs (ncRNAs).
MicroRNAs (miRNAs).
Piwi interacting RNAs (piRNAs).
Long non-coding RNAs (lncRNAs).
Tubulin cofactor A (TBCA).
Bisphenol A (BPA).
Differential DNA methylation regions (DMRs).
Differential histone retention sites (DHRs).

Author Contributions

MBM—Writing and editing manuscript.
EN—Writing and editing manuscript.
MKS—Funding acquisition, writing and editing manuscript.

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Challenging dogmas: How transgenerational epigenetics reshapes our views on life

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Abstract

The emergence of the field of transgenerational epigenetics inheritance (TEI) has profoundly reshaped our understanding of the relationships between environment, soma, and germ cells as well as of heredity. TEI refers to the changes in chromatin state, gene expression, and/or phenotypes that are transmitted across several generations without involving changes to the DNA sequences. TEI has direct connections with, and feeds from, the fields of molecular biology, genetics, developmental biology, and reproductive biology, among others. However, the expansion of TEI-related research, has profoundly reshaped boundaries within each field and often led to the erosion of theories and concepts considered as tenets of biology. We first explore how the molecularization of biology has shifted the definition of epigenetics to include the notion of heredity and how epigenetics has refined our understanding of the central dogma of biology. The demonstrated transfer of environmental information from soma to germ cell through extracellular vesicles and subsequent alteration of health outcomes in offspring has put a definite end to the long-held principle of the Weismann barrier. TEI has also simultaneously led to the revival of the inheritance of acquired characteristics while further eroding the concept of an epigenetic "blank slate" in mammals. Using an historical framework, and via the exploration of central studies in the field, in this perspective article, we will draw a compelling argument for the revolutionary aspect of TEI in biology.

KEYWORDS

transgenerational inheritance, TEI, epigenetics

1 | INTRODUCTION

Transgenerational epigenetics inheritance (TEI) can be defined as changes in chromatin state, gene expression, and/or phenotypes that are transmitted across several generations without involving changes to the DNA sequences. This subfield of epigenetics has grown tremendously in the last decade due to major findings in a variety of model systems that have helped reshape the molecular understanding of heredity. As we will argue, TEI has also been able to find its place as a field of study because of the erosion of long-held views and dogmas in biology. Together TEI and epigenetics have contributed to the overwhelming amount of evidence for the Weismann barrier to be laid to rest, and have encouraged the revisitation and refinement of concepts, such as the central dogma of molecular biology. Here, we will start by framing the discourse through the lens of Waddington's understanding of the relationship between genotype and phenotype and explore how progress in the field of TEI and research on lived experiences,

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such as stress and environmental exposure have brought renewed attention to the concept of soft inheritance.

2 | WADDINGTON AND THE DEFINITION OF THE EPIGENOME

In his seminal 1942 publication, "The Epigenotype," Conrad H. Waddington described the study of mechanisms that exist between genotypes and the phenotypic effects they bring about. Specifically, Waddington provided a prototype for a definition of epigenetics by stating that it refers to "the causal mechanisms at work [...] linked together in a network [...] by which the genes of the genotype bring about phenotypic effects." (Waddington, 2012). Thus, Waddington theorized, in a way akin to modern systems biology, that the developmental processes existing in this space form a network such that early perturbations could funnel to downstream consequences on phenotype. Waddington mentions the example of the "gray-lethal" that prohibits the absorption of bone which has repercussions on other body systems (Grunberg, 1938). For the teeth in particular, impaired coordination and delayed calcification prevents the proper mastication of food. The lack of bone absorption also creates added pressure on nerves in the lower jaw and prevents the animal from taking liquids, leading to starvation and death. Thus, the spontaneous "gray-lethal" mutation of the gene disrupts bone absorption which in turn leads to neuralgic pain and death. From our current perspective, this example has very little to do with our present use of the term epigenetics.

Waddington's concept of the epigenotype was deeply influenced by the current of thoughts in embryology and the debates around the nature and position of the components responsible for carrying out an organism's developmental plan (Felsenfeld, 2014). Obviously, since Waddington's original framework, and with the molecularization of biology, the definition of epigenetics has shifted from its focus on genotype-phenotype interactions towards the stability of expression states and cellular inheritance. It was verified that DNA methylation sites were palindromic, and that DNA methyltransferases were responsible for methylation of unmodified or hemi-methylated DNA (reviewed in Goll & Bestor, 2005). Importantly, these DNA methylation marks are copied on daughter strands after replication, which results in the transmission of the methylated state to future cellular generations (Goll & Bestor, 2005). This finding has led to the insertion of heritability into the definition of epigenetics and, in 1996, Riggs reinterpreted epigenetics as "the study of mitotically or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence" which has become the near standard definition of epigenetics (Riggs & Porter, 1996). Thus, the shift in definition of epigenetics opened a space for TEI to grow as an area of investigation: if the epigenetic marks are heritable then what molecular mechanisms exist that allow them to be transmitted? If some epigenetic marks are stable over several or many generations, and considering that DNA mutations are reversible, then what truly separates DNA-based heredity from TEI?

3 | REVISITING THE CENTRAL DOGMA OF MOLECULAR BIOLOGY

As the definition of epigenetics shifted, so did the central dogma of biology. First defined by Francis Crick in 1957, the central dogma explains the direction of flow for genetic information in our cells. In its purest form, it translates to "DNA makes RNA, and RNA makes protein." (Crick, 1958, 1970). Crick also specified that "the transfer of information from nucleic acid to nucleic acid, or from nucleic acid to protein may be possible, but transfer from protein to protein, or from protein to nucleic acid is impossible." However, epigenetic pathways conflict with this rigidity and have transformed our interpretation of the genome at large. By altering the patterns of DNA methylation and that of histone modifications and noncoding RNAs (ncRNAs), the epigenetic machinery of writers, erasers, and readers directs in what cellular context, and when, specific genes should be expressed (Gillette & Hill, 2015). Similarly, messenger RNAs (mRNAs) are also subject to posttranslational modification to both modulate gene expression and control their metabolism (Frye et al., 2018). For example, established by a multiprotein writer complex, N6-methyladenosine (m6A) is the most abundant modification of eukaryotic mRNAs and directly and indirectly affects the binding of various reader proteins, which either target mRNAs for degradation or translation (Yue et al., 2015). Recent studies have focused on the role of m6A during development for embryonic and adult stem cell differentiation (Roundtree et al., 2017). Specifically, m6A marks transcripts that contain vital developmental regulators to ensure proper transcriptome switching during cell fate transitions. Other work indicates that m6A could interact with chromatin regulatory complexes and long noncoding RNAs to influence transcription as well (Patil et al., 2016).

Finally, ncRNAs also play a central role in the epigenetic regulation of chromatin states. In the fission yeast *S. pombe*, small interfering RNA targets and silences the repetitive pericentromeric region (Reinhart & Bartel, 2002). To accomplish this, three interacting protein complexes are involved. The RITS complex first determines the genomic location of the heterochromatin (Li et al., 2009). Then, the RNA-directed RNA polymerase complex (RDRC) amplifies small RNAs from the selected locus (Motamedi et al., 2004). Finally, the Clr4-containing complex establishes the heterochromatic mark (Zhang et al., 2008). Counterintuitively, some level of transcription is necessary to maintain silencing of the heterochromatic locus: the pericentromeric transcripts initially recognized by the RITS complex are synthesized into double-stranded RNA and processed into new small interfering RNAs that load additional RITS complexes, creating a reinforcing loop (Colmenares et al., 2007). The RITS complex also recruits other writers to promote histone deacetylation and H3K9 methylation (Moazed, 2009). In another well-understood example, in female mammals, the long noncoding RNA Xist plays a vital role in X chromosome inactivation. Xist-mediated silencing requires the presence of Xist A-repeats, which are structurally conserved (Wutz et al., 2002). As Xist spreads, it

directly recruits polycomb repressive complex 2 that mediates the tri-methylation of H3K27 (Engreitz et al., 2013).

Thus, while sequence-based information may follow the unidirectional nature of Crick's central dogma, epigenetic mechanisms significantly add complexity to, and transform, the absolute linear schematic of the central dogma to resemble a molecularized version of Waddington's network.

4 | THE END OF THE WEISMANN BARRIER

While other concepts and dogmas have been refined by epigenetics and TEI over time, none has been affected as much as the Weismann barrier (Bline et al., 2020). The Weismann Barrier refers to the unidirectional and irreversible flow of developmental potential from germline to soma and has informed discussion about evolutionary genetic inheritance for much of the past century. However, recent transgenerational epigenetic inheritance research has refuted core tenets of the theory and has indicated a larger role for environmental influence than previously held. The concept of a barrier originates from Weissman's germ plasm theory, which he used to justify how organisms remain relatively constant through generations but adapt to their environment over evolutionary time (Weismann, 1893). He argued that after fertilization, a zygote had two paths for replication. The first choice was "embryonic" cell division, where daughter cells received some parts of the nuclear content. The resultant "idioplasm" gave rise to bodily tissues of the organism. The second choice was "ordinary" cell division, where the daughter cell received all nuclear contents of the parent. Here, a "reserve" germ plasm was produced, to be further transmitted to new generations. Importantly, Weismann postulated that idioplasm could under no circumstances be reconstructed into germ plasm. Therefore, any bodily changes brought about by external stressors during an organism's lifespan would not be passed on to their children. This inability of idioplasm to revert to germ plasm and be inherited by offspring necessitated the existence of a barrier that prevented interaction between the two, which was later named the Weismann Barrier.

While the Weismann Barrier hinges on the separation of germ cells and somatic cells, recent studies have demonstrated that the two interact and exchange epigenetic information to create an additional source of heredity shaped by the parental environment. Multiple groups have now demonstrated and characterized the transfer of small RNAs between somatic and germline cells in mammals (Bohacek & Mansuy, 2015; Jawaid et al., 2018). For instance, in mice, metabolic tracing successfully verified that caput epididymosomes transport small RNA cargos initially synthesized in the epididymal epithelium to spermatozoa (Sharma et al., 2018). The content of these cargos is altered based on the various stress conditions or dietary perturbations encountered by the father preconception. With regard to models of stress, zygotes injected with sperm RNAs from males subjected to the MSUS paradigm – maternal separation coupled with unpredictable maternal stress – give rise to offspring that exhibit depression-like symptoms. These offspring display an increased tendency to remain afloat during forced swim tests, which has been established as an accurate measure of passive coping (Sharma & Rando, 2014). Remarkably, in other studies, the same increase in floating time can be elicited transgenerationally (until the F3) when MSUS is applied during gestation (van Steenwyk et al., 2018). The ability for sperm to carry experiential information to the offspring is not limited to stress-related cues. Offspring generated from mice raised on high-fat diets show impaired glucose metabolism and a small 30–40 nt sperm RNA (tsRNAs) fractions was shown to be the primary mediator of such effects (Chen et al., 2016). Interestingly, the tsRNA cargo requires itself epigenetic modification as demonstrated by the dependence of the transmission of impaired glucose metabolism on active tRNA methyltransferase DNMT2 (Zhang et al., 2018), again highlighting the complex interplay and layering of epigenetic information.

In a recent elegant set of experiments, van Steenwyk and colleagues showed that early life trauma in mice and humans raises the levels of specific serum lipid metabolite species that have the ability to activate PPARγ in sperm. Interestingly, injection in male mice of serum from mice subjected to MSUS or of a PPARγ agonist is sufficient to recreate the MSUS-induced glycemic deregulation in offspring (van Steenwyk et al., 2020). Together, these tantalizing developments in the field of TEI go exactly against the concept of the Weismann barrier: external influences on the soma can be physically transferred to the germ cells and be inherited by the offspring.

5 | THE INHERITANCE OF ACQUIRED CHARACTERISTICS AND THE RETURN OF SOFT INHERITANCE

While new advances in the field of TEI are putting some old concepts to rest, they are concomitantly reviving others that had largely been abandoned. In interesting ways, TEI resembles the concepts underlying the theory of inheritance of acquired characteristics, a common belief among eighteenth and nineteenth-century naturalists until it was mostly discarded for Darwin's views of evolution and the Mendel, Boveri-Sutton, Morgan principles of heredity. The theory of inheritance of acquired characteristics, or the ability for acquired characteristics during an organism's lifetime to be passed to their offspring, has long been associated with Jean Baptiste-Lamarck, even though most of the underlying principles of the theory were not his own (Burkhardt, 2013). Instead, Lamarck simply delineated specific conditions necessary for it to occur. Unlike his contemporaries, he argued that organisms took on new forms because of environmentally-shaped habits they acquired and not vice versa. To support this, Lamarck described how the webbed feet of the wading bird resulted from its continual exposure to sinking in mire when searching for prey. The bird will get in the habit of contracting its legs to elongate itself, and after successive generations this habit will be biology encoded in the species.

Conversely, he postulated that constant disuse of an organ or part would cause it to deteriorate until it finally disappears.

While these ideas were eventually dismissed in favor of Darwinian views of evolution, the growth of TEI has signaled the return of a modernized version of Lamarckism: soft inheritance. Soft inheritance, bolstered by a growing body of evidence that variation in epigenetic states is not random but rather initiated and guided by the environment, posits that environment cues can influence hereditary information. While it remains a challenge to comprehensively identify the mechanisms of epigenetic inheritance from other environmental cues, the aforementioned studies indicate that parental lived experience, such as stress can impact the health of descendants spanning several generations.

Interestingly, for soft inheritance and TEI to align at the molecular level, yet another concept needed refining: the ability of early mammalian zygotes and early germ cells, termed primordial germ cells, to profoundly reprogram their epigenome, thereby producing an epigenetic "blank slate" (Messerschmidt et al., 2014). However, this "blank slate" specifically refers to the waves of DNA demethylation during early development while other epigenetic marks do not see reprogramming to a similar extent. Furthermore, even DNA methylation is not completely lost, it is reduced to around 10%, instead being actively maintained at critical genomic regions, such as repetitive elements (Tang et al., 2016).

The ability of environmental information to cause detectable and heritable alterations to the epigenome has now been described in a multitude of model organisms and involve all three types of epigenetic modifications. For example, in nematodes, the plastic component Bisphenol A causes transgenerational reproductive defects that are dependent on two histone modifications, H3K9me3 and H3K27me3 (Camacho et al., 2018; Weinhouse et al, 2018). Endocrine disruptors also lead to detectable transgenerational changes in DNA methylation in Zebrafish (Akemann et al., 2020) as well as in rats (Gillette et al., 2018). In the latter study, the authors examined the number of differentially methylated regions (DMRs) occurring in sperm and specific brain nuclei involved in stress response and social behavior caused by in utero exposure to two endocrine disruptors Vinclozolin and the polychlorinated biphenyl mixture A1221. They observed a high number of DMRs in the sperm, and to a lesser extent also in the brain regions, of male F1 and F3 progeny (Gillette et al., 2018). Remarkably, there was substantial overlap in the number and direction (hyper vs hypo-methylation) of the DMRs in sperm between F1 and F3 generations. This study opens exciting avenues to investigate whether in mammals, DMRs are maintained "as is" across generation or whether a relay-mechanism exist that would involve cross-talks with other epigenetic marks or other mechanisms.

6 | CONCLUSION

While the examples we have examined in this paper have been confined to stress, diet, and environmental chemicals, the field of TEI encompasses many other types of environmental information. However, the field still suffers from a relative fragmentation of the research that forms it: fragmentation by model organism, type of epigenetic modification, environmental cue, and sex. Questions of the applicability of the model organisms to humans are also not to be ignored, and continued examination of human cohorts, such as the often-cited example of the Dutch Hunger Winter study (Stein et al., 1975), while difficult, will provide critical proof of the relevance of findings in other model systems. Nonetheless, advances in the fields of epigenetics and TEI have already reshaped, or put to rest, numerous views previously considered as rules and/or central to biology.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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"Systems Biology of Reproduction"

Spring 2024 (Even Years) – Course Syllabus
Biol 475/575 Undergraduate/Graduate (3 Credit)
SLN: (475) – 06763, (575) – 06764
Time - Tuesday and Thursday 10:35 am-11:50 am
Course Lectures in person and recorded on Canvas/Panopto and Discussion Sessions live in person and on WSU Zoom for all campuses (Hybrid Course)
Room – CUE 418
Course Director – Michael Skinner, Abelson Hall 507, 335-1524, skinner@wsu.edu
Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, nilsson@wsu.edu
Learning Objective -
Current literature based course on the Systems Biology of Reproduction. Learning Systems approaches to the biology of reproduction from a molecular to physiological level of understanding.
Schedule/Lecture Outline –

January	9 & 11	Week 1	Systems Biology Introduction
	16 & 18	Week 2	Molecular/ Cellular/ Reproduction Systems
	23 & 25	Week 3	Sex Determination Systems
Jan /Feb	30 & 1	Week 4	Male Reproductive Tract Development & Function
February	6 & 8	Week 5	Female Reproductive Tract Development & Function
	13 & 15	Week 6	Gonadal Developmental Systems Biology
	20 & 22	Week 7	Testis Systems Biology
	27 & 29	Week 8	Ovary Systems Biology
March	5 & 7	Week 9	Epigenetics and Transgenerational Gonadal Disease
	11 – 15	**Week 10**	**Spring Break**
	19 & 21	Week 11	Gametogenesis/ Stem Cells/ Cloning
	26 & 28	Week 12	Hypothalamus-Pituitary Development & Function
April	2 & 4	Week 13	Reproductive Endocrinology Systems
	9 & 11	Week 14	Fertilization & Implantation Systems
	16 & 18	Week 15	Fetal Development & Birth Systems
	23 & 25	Week 16	Assisted Reproduction/Contraception
Apr/May	30 & 2	Week 17	Exam or Grant Review

Spring 2024 – Systems Biology of Reproduction
Lecture Outline – Epigenetics and Transgenerational Reproductive Disease
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
March 5, 2024
Week 9

Epigenetics and Transgenerational Reproductive Disease

- Environmental Induced Pathology
- Transgenerational Disease Phenotype
- Compound Specificity
- Epigenetic Mechanism
- Epimutations and Exposure Specificity
- Transgenerational Transcriptome
- Transgenerational Testis Disease
- Transgenerational Ovary Disease
- Broader Impact

REQUIRED READING

Ben Maamar M, Nilsson EE, Skinner MK. Epigenetic transgenerational inheritance, gametogenesis and germline development. Biol Reprod. 2021 Sep 14;105(3):570-592.

Wang HD, Allard P. Challenging dogmas: How transgenerational epigenetics reshapes our views on life. J Exp Zool A Ecol Integr Physiol. 2022 Jan;337(1):70-74.

Spring 2024 – Systems Biology of Reproduction
Discussion Outline – Epigenetics and Transgenerational Reproductive Disease
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
March 7, 2024
Week 9

Epigenetics and Transgenerational Reproductive Disease

Primary Papers:

1. Kubsad, et al. (2019) Scientific Reports 9 :6372
2. Sadler-Riggleman, et al. (2019) Environmental Epigenetics 5(3) dvz013
3. Beck, et al. (2021) Epigenetics & Chromatin 14:6

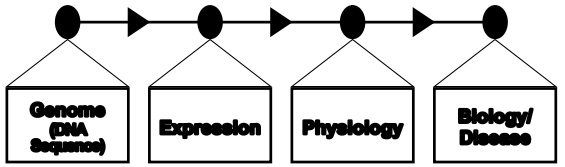
Discussion

Student 11: Reference 1 above
- What transgenerational reproductive phenotypes were observed?
- What is glyphosate and effects on sperm?
- What is generational toxicology?

Student 1: Reference 2 above
- What environmental exposures were used?
- What was the Sertoli cell effects observed?
- What basic information on testis disease was obtained?

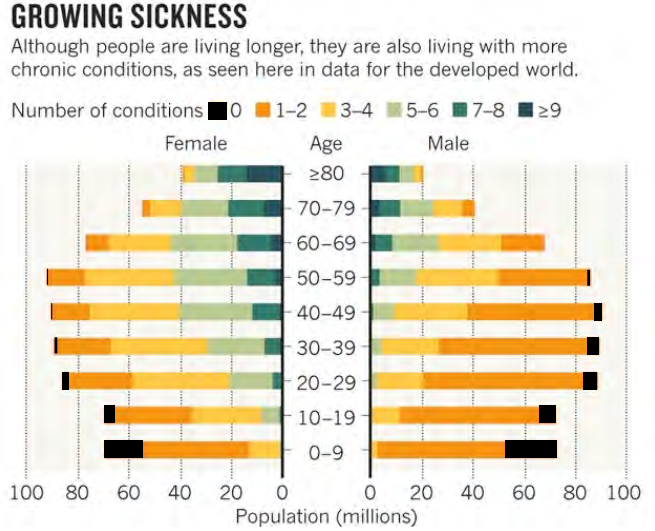
Student 2: Reference 3 above
- What technology and epigenetic analysis was used?
- What differential epigenetic regions were observed?
- What transgenerational integration of epigenetic processes was observed?

GENETIC DETERMINISM



ENVIRONMENTAL IMPACT ON BIOLOGY

- **Regional Disease Frequencies**
- **Low Frequency of Genetic Component of Disease**
- **Increases In Disease Frequencies**
- **Identical Twins and Variable Disease Frequency**
- **Environmental Exposures and Disease**
- **Evolution and Rapid Induction**



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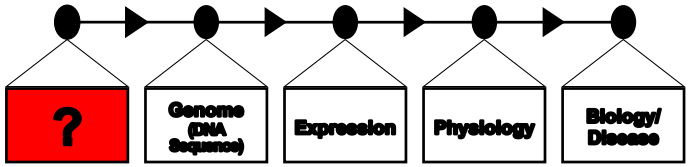
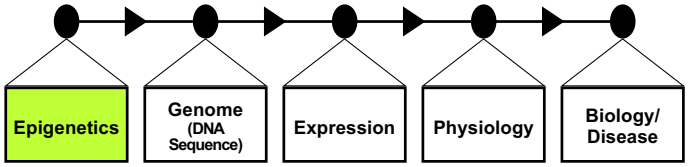
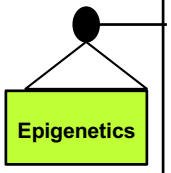
GENETIC DETERMINISM



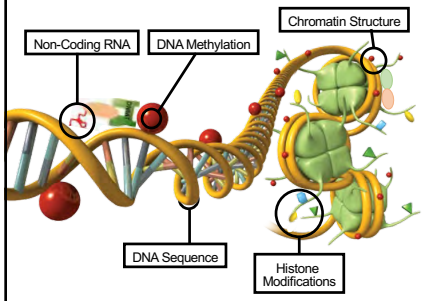
EPIGENETIC SOLUTIONS TO GENETIC DETERMINISM FAILURES





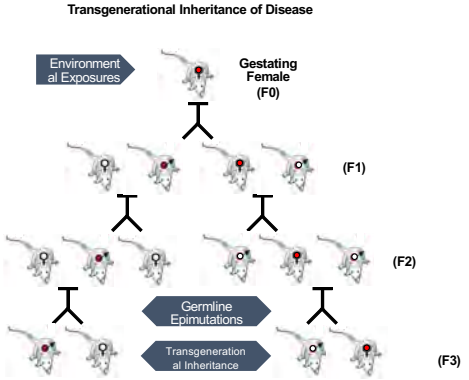
EPIGENETICS

Molecular factors/processes around the DNA that regulate genome activity, independent of DNA sequence, and are mitotically stable

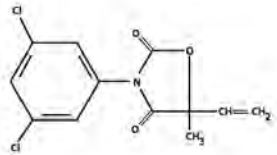


EPIGENETIC MECHANISMS AND MARKS

- **DNA Methylation**
- **Histone Modifications**
- **Chromatin Structure**
- **Non-coding RNA**
- **RNA methylation**

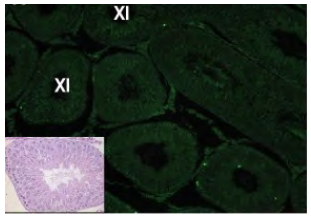


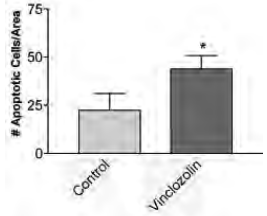
Model Endocrine Disruptor: Vinclozolin

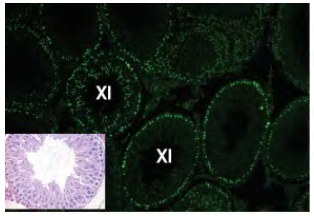


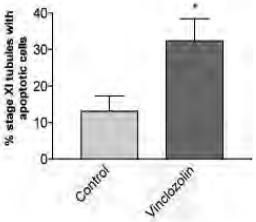
- Vinclozolin is a systemic fungicide (e.g. Wine Industry)
- Vinclozolin and its metabolites are anti-androgenic
- Late embryonic/early postnatal exposure causes abnormal reproductive tract development and gonadal function

Vinclozolin Effects on Spermatogenic Cell Apoptosis

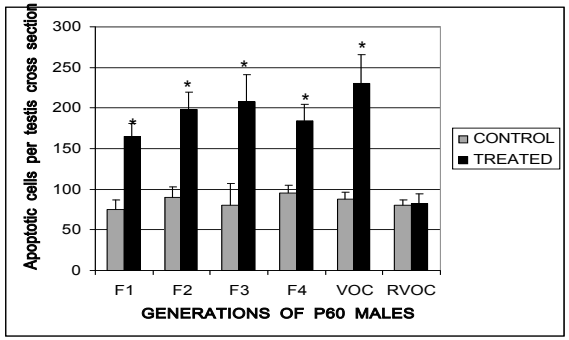








Germ Cell Apoptosis



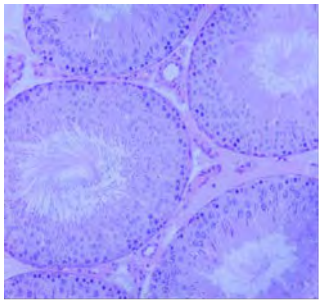
* P<0.05

Vinclozolin

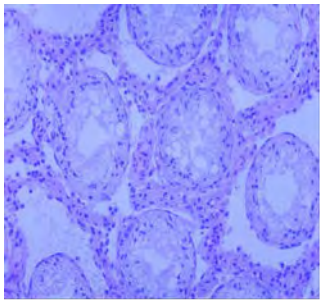
Complete Male Infertility (10%)

F3 Generation Males

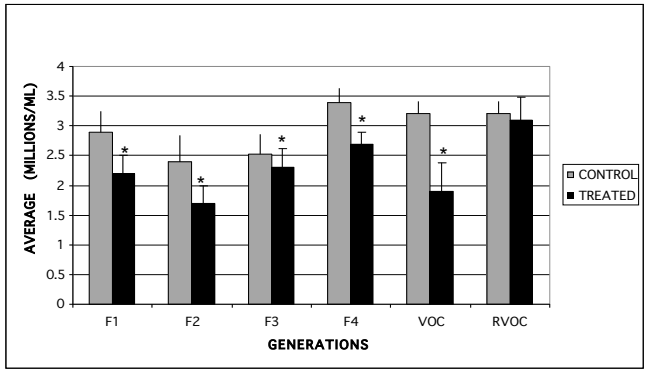
Control



Vinclozolin



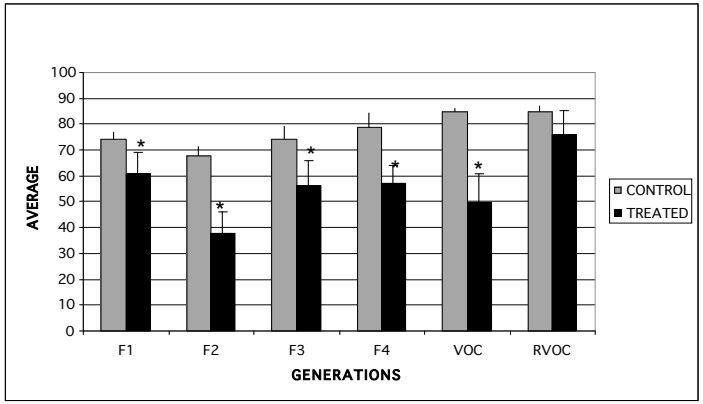
Sperm Concentration P60-P150



* P<0.05

Vinclozolin

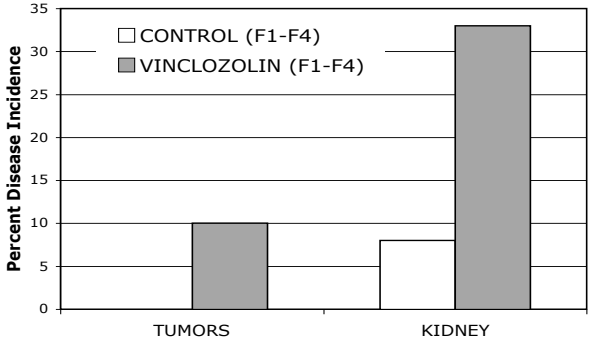
Sperm Motility P60-P150

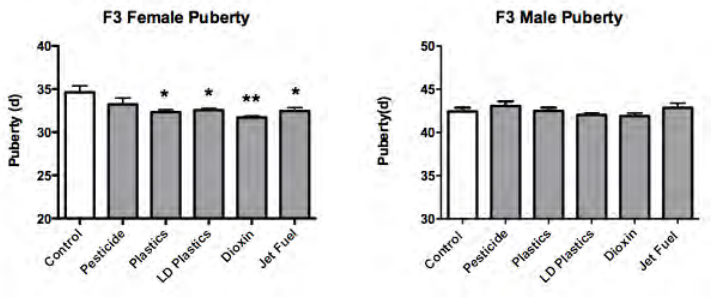


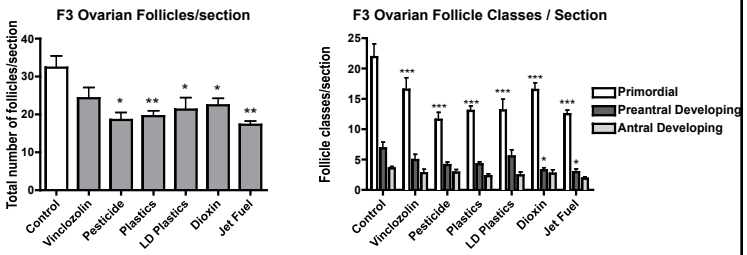
* P<0.05

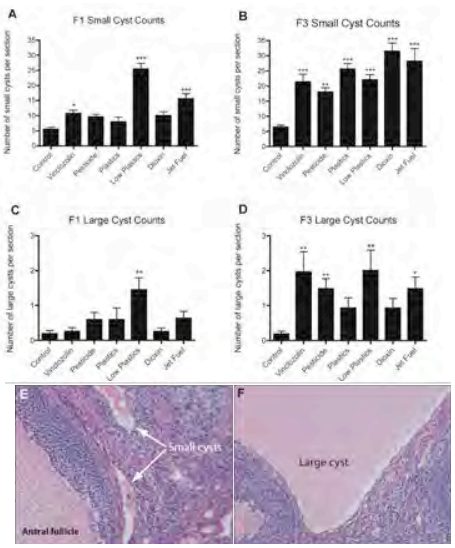
Vinclozolin

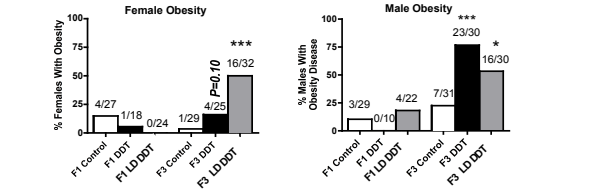
Transgenerational Disease in Female F1-F4

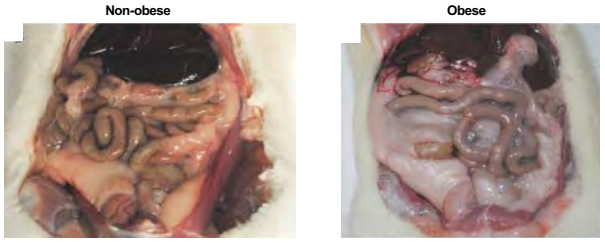




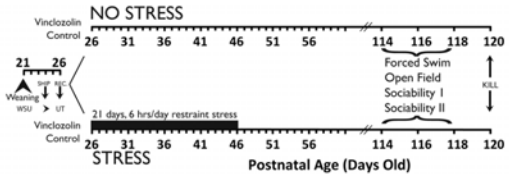




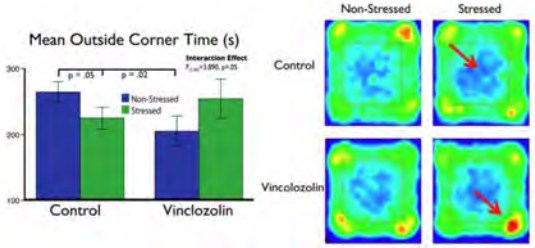




Epigenetic Transgenerational Inheritance and Stress Response



Open Field Anxiety Stress Response



TRANSGENERATIONAL DISEASE ETIOLOGY

MALE	FEMALE	
▪ Spermatogenic Defect (>90%)	▪ Kidney disease (~30-40%)	▪ Pre-eclampsia-like during late pregnancy (~10%)
▪ Male infertility (complete ~10%, severe 20%)	▪ Increase in mammary tumor formation (~10-20%)	▪ Premature Ovarian Failure POF (>90%)
▪ Prostate disease (~50%)	▪ Behavior (Mate Preference, Anxiety, & Stress) (>90%)	▪ Ovarian Polycystic Ovarian Disease (>90%)
	▪ Pubertal Abnormalities (~25%)	▪ Female Premature Pubertal Onset (>90%)
	▪ Obesity (~10-50%)	▪ Parturition (birth) defects
	▪ Lean (~10-20%)	
	▪ Immune (~20%)	

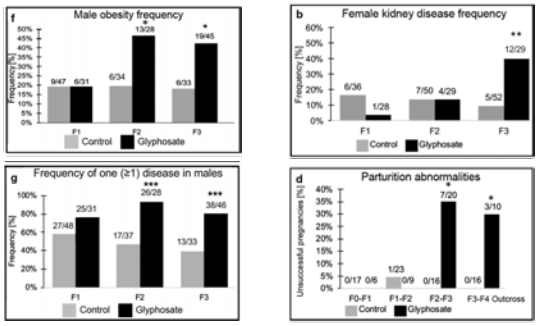
Compound Specificty

Other Endocrine Disruptors
And Environmental Compounds

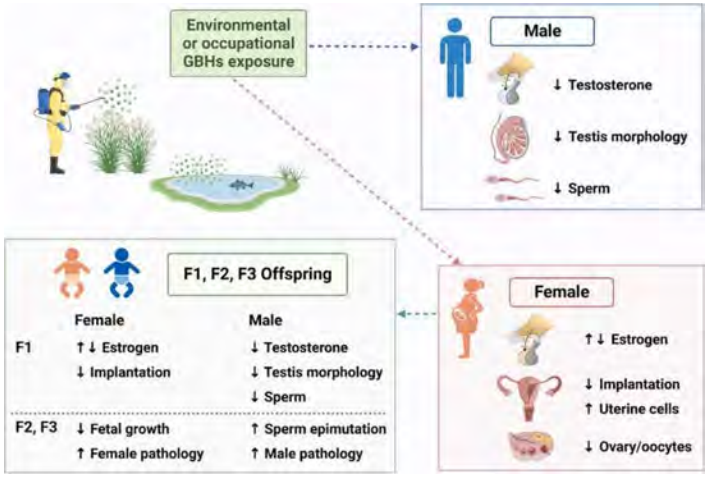
Environmental Compound Specificity

(Exposure Groups)	(Direct) F1	F3 (Transgenerational)
A. Vinclozolin [agricultural fungicide]	Yes	Yes
B. Flutamide [anti-androgenic pharmaceutical]	Yes	No
C. TCDD/Dioxin (industrial pollutant)	Yes	Yes
D. Plastics Compounds [Bisphenol-A BPA, Phthalate-DEHP & DBP]	Yes	Yes
E. Jet Fuel [JP8] (Hydrocarbon Mixture)	Yes	Yes
F. Pesticide & Insect Repellent [Permethrin & DEET]	No*	Yes
G. DDT (pesticide)	Yes	Yes
H. Methoxychlor (pesticide, replace DDT)	Yes	Yes
I. Mercury (Industrial pollutant)	Yes	Yes
J. Atrazine (agricultural herbicide)	No*	Yes
K. Glyphosate (pesticide herbicide)	No*	Yes

Assessment of Glyphosate Induced Epigenetic Transgenerational Inheritance of Pathologies and Sperm Epimutations: Generational Toxicology.
Kubsad D, Nilsson EE, King SE, Sadler-Riggleman I, Beck D, Skinner MK.
Sci Rep. 2019 Apr 23;9(1):6372.

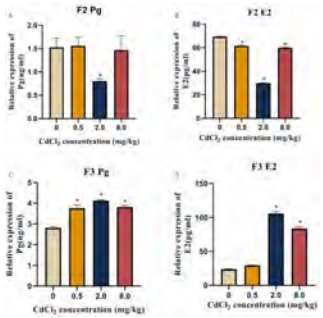


Effects of glyphosate-based herbicides and glyphosate exposure on sex hormones and the reproductive system: From epidemiological evidence to mechanistic insights.
Tajai P, Pruksakorn D, Chattipakorn SC, Chattipakorn N, Shinlapawittayatorn K.
Environ Toxicol Pharmacol. 2023 Sep;102:104252

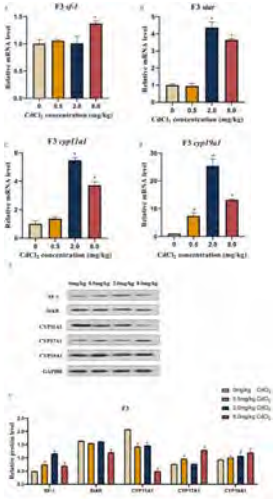


Maternal genetic intergenerational and transgenerational effects on hormone synthesis in ovarian granulosa cells of offspring exposed to cadmium during pregnancy.
Luo L, Li J, Sun Y, Lv Y, Liu J, Li Y, Zhang C, Zhang W.
Ecotoxicol Environ Saf. 2023 Sep 15;263:115278.

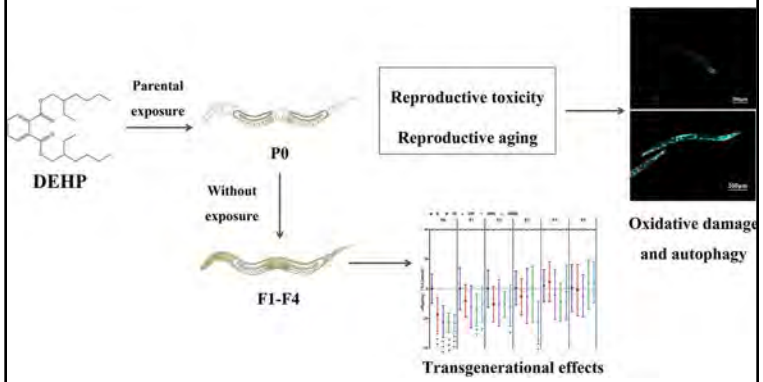
Serum levels of E_2 and Pg in adult female rats of the F2 and F3 generations.



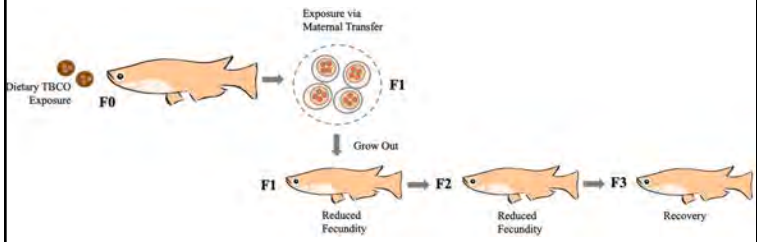
Expression levels of E_2 and Pg synthesis-related genes in the ovarian GCs of F2 adult female rats.



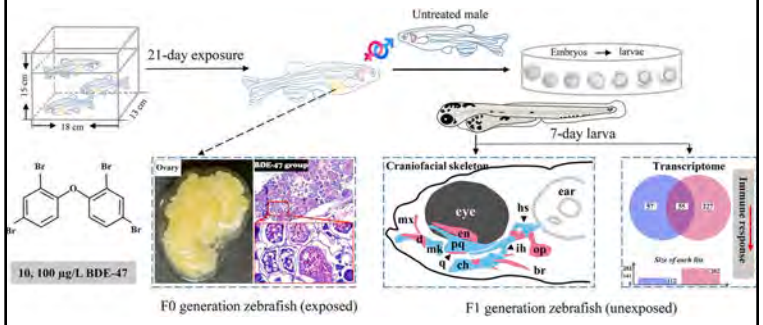
Di(2-ethylhexyl) phthalate induces reproductive toxicity and transgenerational reproductive aging in Caenorhabditis elegans.
Zhang Y, Li J, Shi W, Lu L, Zhou Q, Zhang H, Liu R, Pu Y, Yin L.
Environ Pollut. 2023 Nov 1;336:122259.



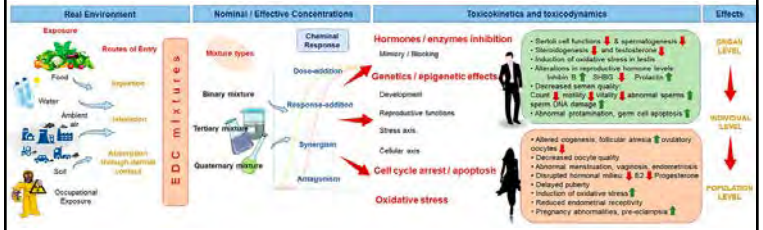
The brominated flame retardant, 1,2,5,6-tetrabromocyclooctane (TBCO), causes multigenerational effects on reproductive capacity of Japanese medaka (Oryzias latipes).
Devoy C, Raza Y, Kleiner M, Jones PD, Doering JA, Wiseman S.
Chemosphere. 2023 Feb;313:137561.



Transgenerational effects of BDE-47 to zebrafish based on histomorphometry and toxicogenomic analyses.
Huang W, Shi X, Zhang Q, Chen Y, Zheng S, Wu W, Luo C, Wu K.
Chemosphere. 2023 Dec;344:140401.



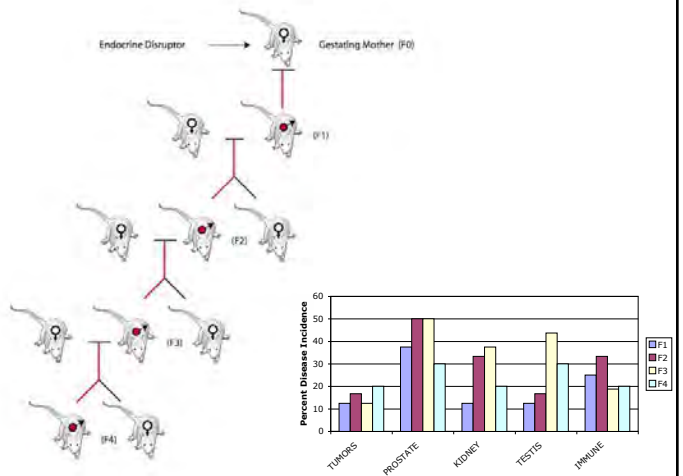
Reproductive toxicity of combined effects of endocrine disruptors on human reproduction.
Dutta S, Sengupta P, Bagchi S, Chhikara BS, Pavlík A, Sláma P, Roychoudhury S.
Front Cell Dev Biol. 2023 May 12;11:1162015.

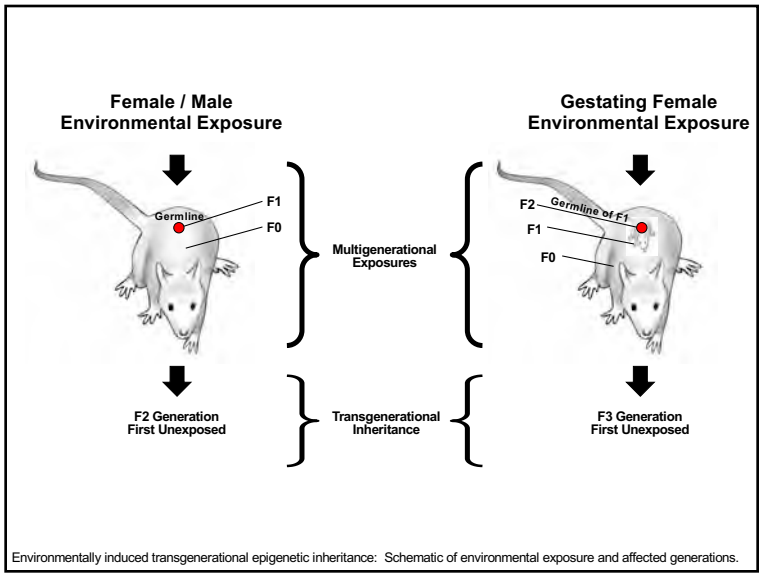


Reproductive dysfunctions by the combined effects of environment and lifestyle derived endocrine disrupting chemicals (EDCs). SHBG, sex hormone-binding globulin; E2, estradiol.

Transgenerational Disease Etiology

- Spermatogenic Defect (>90%)
- Male infertility (complete ~10%, severe 20%)
- Kidney disease (~30-40%)
- Prostate disease (~50%)
- Increase in mammary tumor formation (~10-20%)
- Behavior (Mate Preference,Anxiety&Stress)(>90%)
- Pre-eclampsia-like during late pregnancy (~10%)
- Premature Ovarian Failure POF (>90%)
- Ovarian Polycystic Ovarian Disease (>90%)
- Female Premature Pubertal Onset (>90%)
- Obesity (~10-50%)





Environmentally induced transgenerational epigenetic inheritance: Schematic of environmental exposure and affected generations.

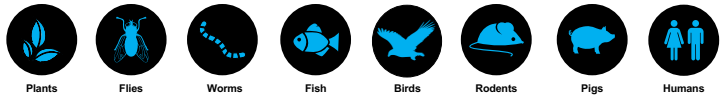
ENVIRONMENTALLY INDUCED EPIGENETIC TRANSGENERATIONAL INHERITANCE

Environmental Toxicants

Vinclozolin (Agricultural Fungicide)	Permethrin & DEET (Insect Repellants)
Methoxychlor (Agricultural Pesticide)	DDT (Pesticide)
Dioxin/TCDD (Industrial Contaminant)	Tributyltin (Industrial Toxicant & Biocide)
Plastic Compounds (BPA & Phthalates)	Hydrocarbons (Jet Fuel)
Atrazine (Herbicide)	Glyphosate (Pesticide / Herbicide)

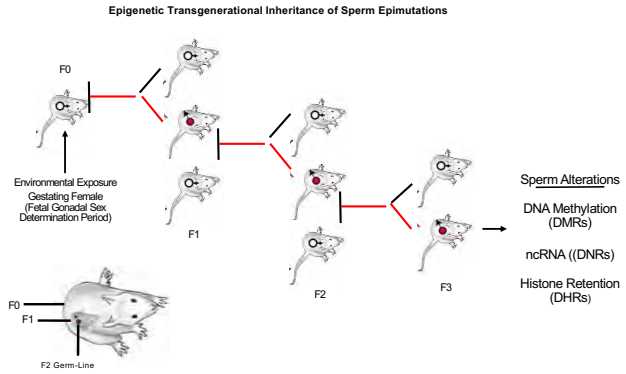
Other Types Exposures

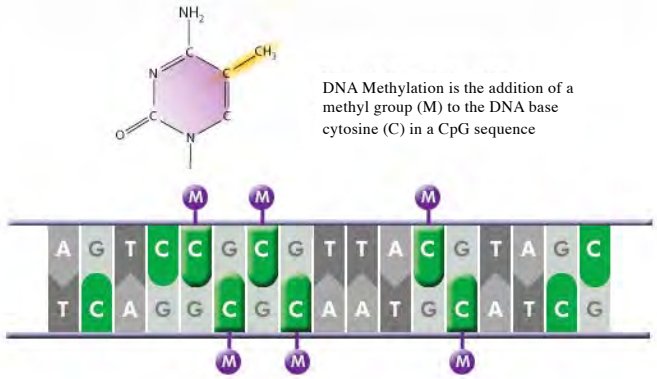
Nutrition (High Fat or Caloric Restriction)	Smoking & Alcohol
Temperature & Drought (Plant Health & Flowering)	Stress Trauma (Behavioral)



Summary

- Transient Embryonic Exposure Effects Adult
  - -Sex Determination Period
  - -Fetal Basis of Disease
- Spermatogenic Fertility Defect & Other Diseases
- Transgenerational Phenotype
  - -WHAT MECHANISM?

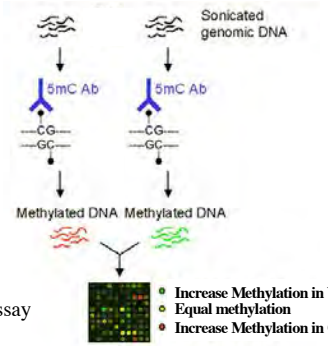




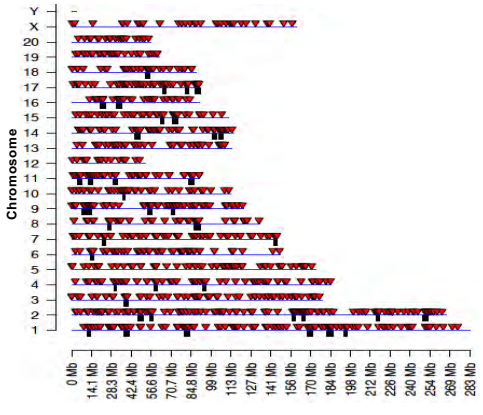
DNA Methylation is the addition of a methyl group (M) to the DNA base cytosine (C) in a CpG sequence

Comparative Methylation, MeDIP Seq, F3 Generation Sperm DNA pools

Vinclozolin Control



MeDIP-Chip Assay
or
MeDIP-Seq



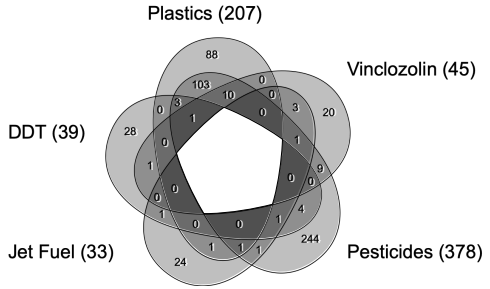
Vinclozolin
F3 Generation
Sperm Genome
Wide Epimutations
(MeDIP-Seq)

1075 p<10-4 DMR Sites

Transgenerational Epimutations & Clusters

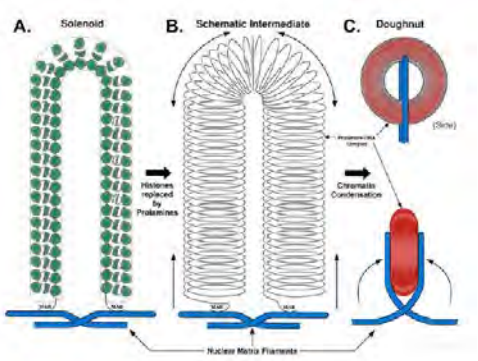
Enviromental Epigenetics 2017

EXPOSURE SPECIFIC TRANSGENERATIONAL (F3) SPERM PROMOTER EPIGENOME ALTERATIONS

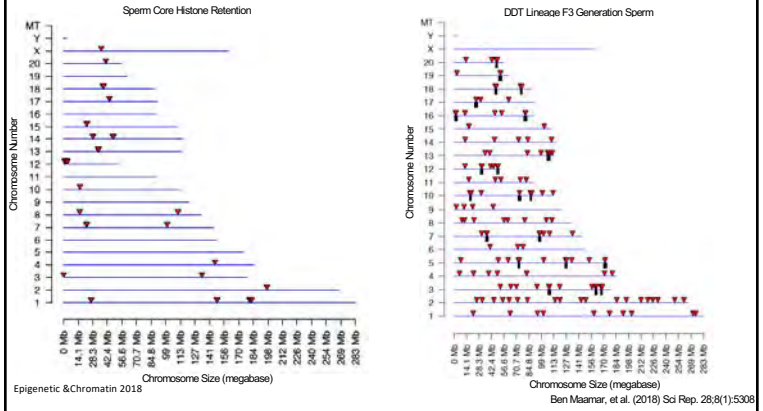


Plos One 2014

Chromatin Structure – Composition and Function during Spermiogenesis
Khochbin, S. and Ward, W.S



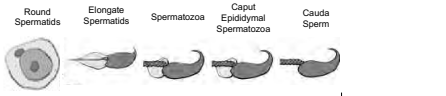
Environmentally Induced New Transgenerational Histone Retention



Epigenetic &Chromatin 2018
Ben Maamar, et al. (2018) Sci Rep. 28;8(1):5308

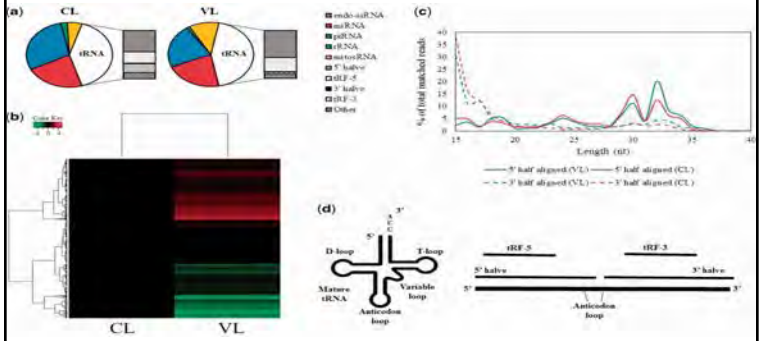
Developmental Origins of Transgenerational Sperm Histone Retention Following Ancestral Exposures
Ben Maamar M, Beck D, Nilsson E, McCarrey JR, and Skinner MK
Developmental Biology (submitted)

Developmental Origins of Sperm Epimutations (DHRs)

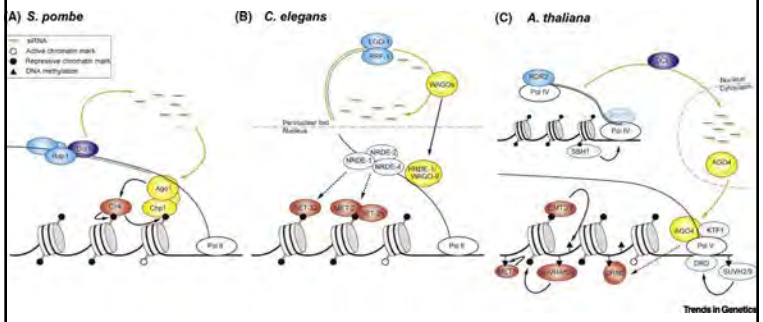


Transgenerational DHRs	DDT	Vinclozolin
Round Spermatids	16	9
Caput	1	1
Cauda	16	7
Total DHRs	33	17

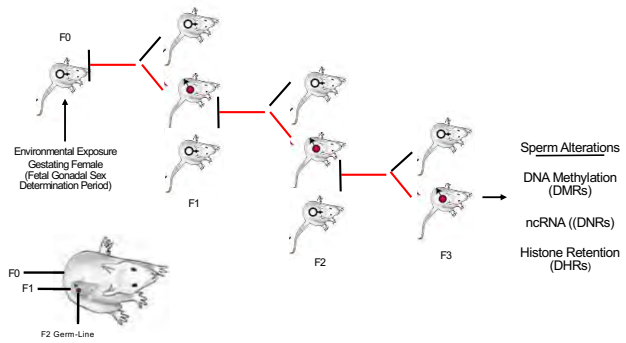
Ancestral vinclozolin exposure alters the epigenetic transgenerational inheritance of sperm small noncoding RNAs (CC)
Andrew Schuster, Michael K. Skinner, Wei Yan
Environmental Epigenetics (2016) 2 (1): 1-15



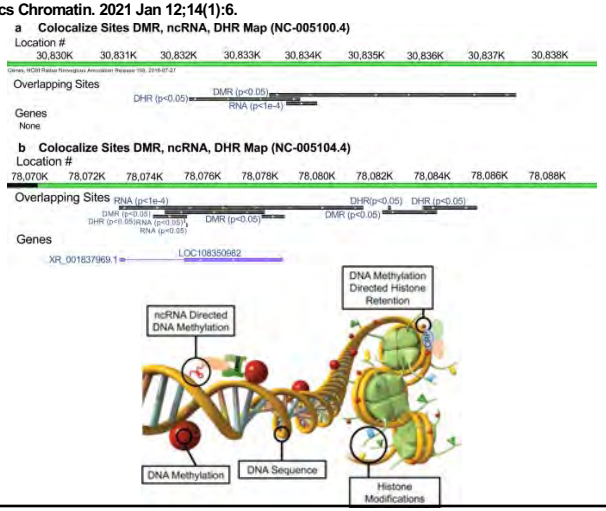
Small RNAs in the Transgenerational Inheritance of Epigenetic Information.
Duempelmann L, Skribbe M, Bühler M.
Trends Genet. 2020 Jan 14. doi: 10.1016/j.tig.2019.12.001. [Epub ahead of print]

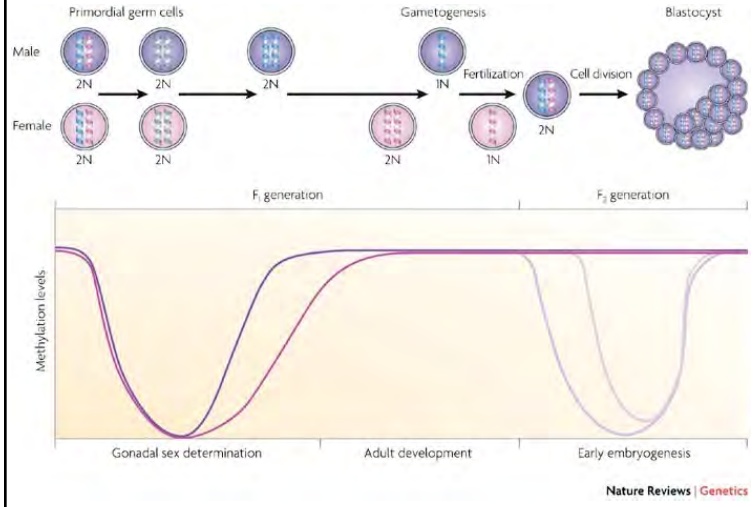


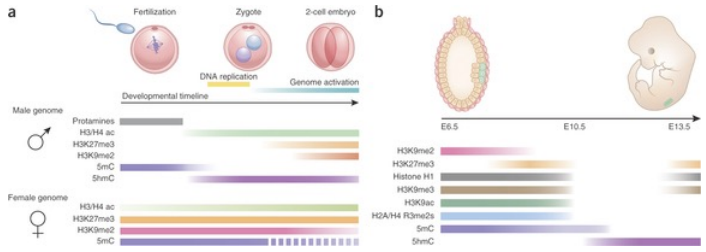
Epigenetic Transgenerational Inheritance of Sperm Epimutations



Integration of sperm ncRNA-directed DNA methylation and DNA methylation-directed histone retention in epigenetic transgenerational inheritance.
Beck D, Ben Maamar M, Skinner MK.
Epigenetics Chromatin. 2021 Jan 12;14(1):6.

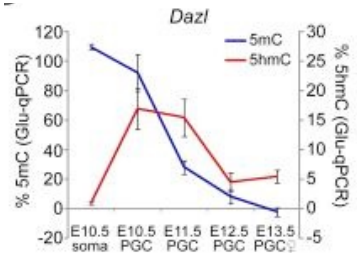




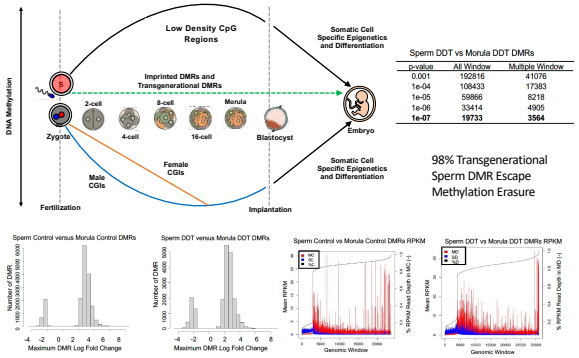


(a) Schematic of global DNA and histone modifications that lead to transcriptional activation of the embryonic genome between the late zygote (paternal genome only) and the 2-cell stage. Gamete genomes undergo different epigenetic programs after fertilization with the paternal genome being mostly subject to epigenetic remodeling at the zygote stage and the maternal genome gradually losing repressive modifications during the subsequent cleavage divisions. (b) Global epigenetic changes during germline development from PGC specification (E6.5) to the mitotic/meiotic arrest at E13.5. Two major reprogramming phases can be distinguished during PGC migration toward the genital ridges (E7.5–E10.5) and upon their arrival into the gonads (E10.5–E12.5).

Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine.
Hackett JA, et al. (2013) Science. 25;339(6118):448-52.



Transgenerational Sperm DMRs and Low Density Genomic CpG Sites Escape Methylation Erasure During Embryonic Development and Epigenetic Inheritance
Ben Maamar M, Wang Y, Nilsson EE, Beck D, Yan W, Skinner MK
Scientific Reports 2023



Sperm DDT vs Morula DDT DMRs

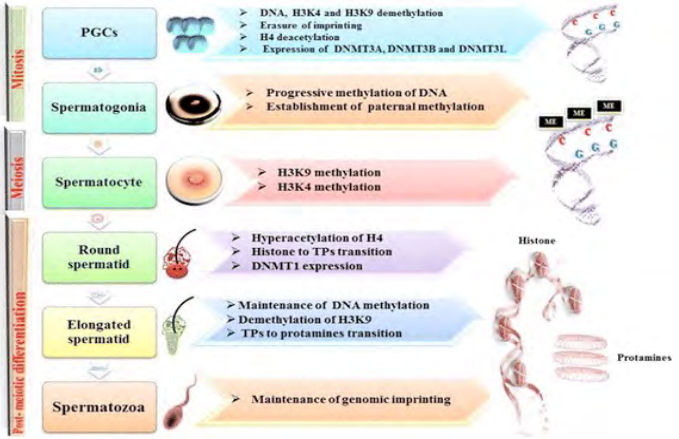
p-value	All Window	Multiple Window
0.001	192816	41076
1e-04	108433	17383
1e-05	59866	8218
1e-06	33414	4905
1e-07	**19733**	**3564**

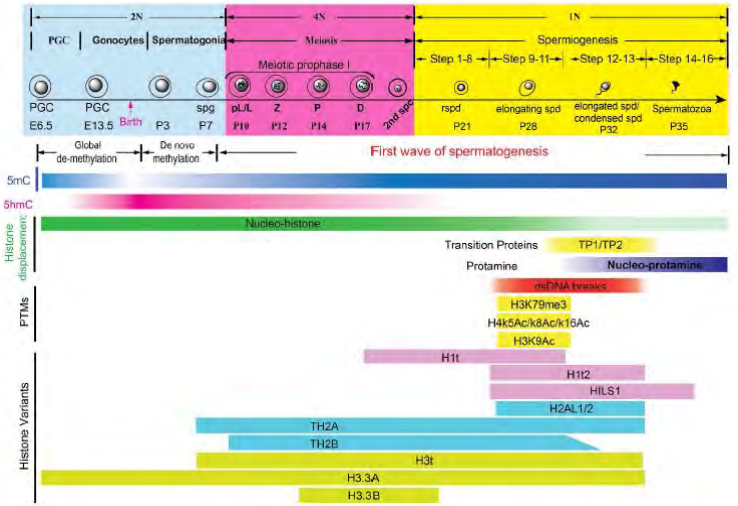
98% Transgenerational Sperm DMR Escape Methylation Erasure

Epigenetic Mechanisms of Gene Regulation

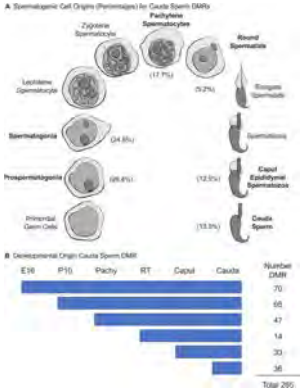
	Spermatogenesis
-DNA Methylation	
-Histone Modification	-Replaced Protamines
-Chromatin Structure	-Condensation
-DNA Organization into Domains (eg Loops)	-Condensation
-Nuclear Compartmentalization (eg nuclear matrix)	-Condensation
-Noncoding functional RNAs	-Silencing (?)

Epigenetics and male reproduction: the consequences of paternal lifestyle on fertility, embryo development, and children lifetime health.
Stuppia L, Franzago M, Ballerini P, Gatta V, Antonucci I.
Clin Epigenetics. 2015 Nov 11;7:120.

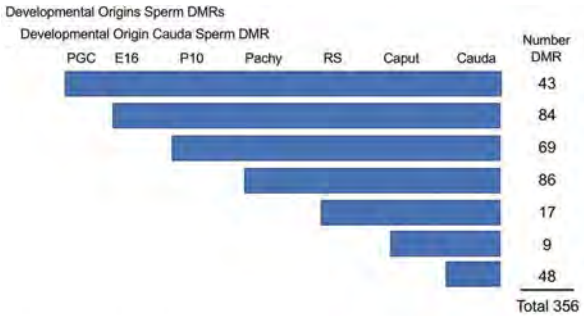




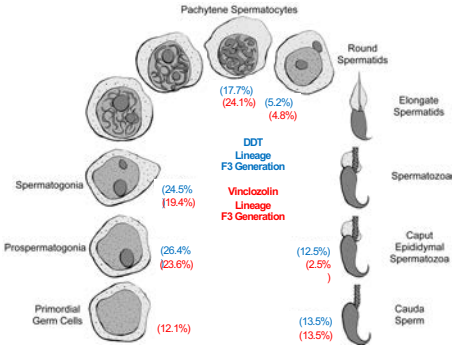
Developmental origins of transgenerational sperm DNA methylation epimutations following ancestral DDT exposure.
Ben Maamar M, Nilsson E, Sadler-Riggleman I, Beck D, McCarrey JR, Skinner MK.
Dev Biol. 2019 Jan 15;445(2):280-293.



Transgenerational sperm DNA methylation epimutation developmental origins following ancestral vinclozolin exposure.
Skinner MK, Nilsson E, Sadler-Riggleman I, Beck D, Ben Maamar M, McCarrey JR.
Epigenetics. 2019 Jul;14(7):721-739.

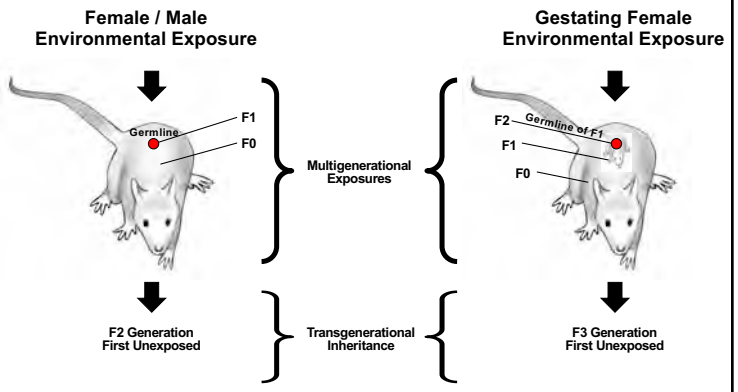


Developmental Origins of Sperm Epimutations (DMRs)



Collaborator- John McCarrey, UTSA

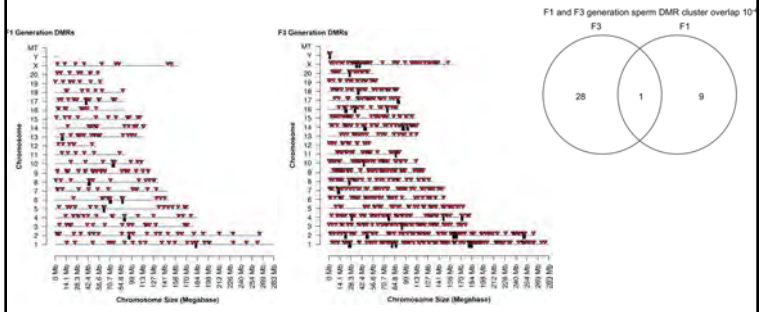
Ben Maamar, et al. (2019) Dev Biol 15;445(2):280-293
Skinner, et al (2019) Epigenetics14(7):721-739



Environmentally induced transgenerational epigenetic inheritance: Schematic of environmental exposure and affected generations.

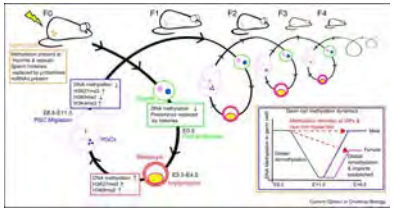
Generational comparisons (F1 versus F3) of vinclozolin induced epigenetic transgenerational inheritance of sperm differential DNA methylation regions (epimutations) using MeDIP-Seq

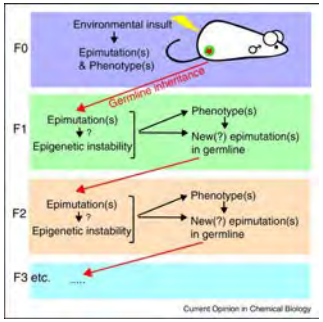
Environ Epigenet. 2017 Jul;3(3). pii: dvx016.
Beck D, Sadler-Riggleman I, Skinner MK.



Unravelling the complex mechanisms of transgenerational epigenetic inheritance

Curr Opin Chem Biol. 2016 Aug;33:101-7.
Blake GE, Watson ED.





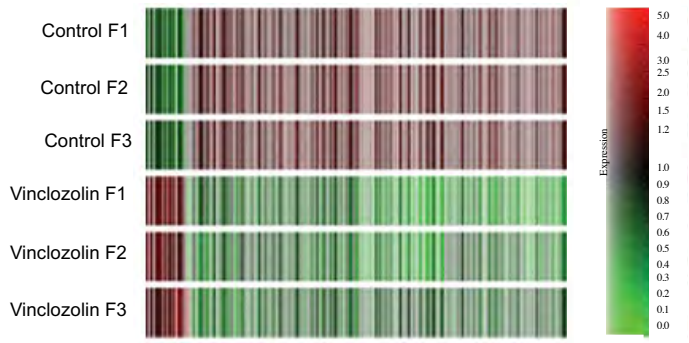
F3 Generation Sperm Epigenome Mapping
(Epimutations)
(Epigenetic Biomarkers for Ancestral Exposures)

Transgenerational Sperm Epigenome Alterations
(>200 differential DNA methylation sites)

Potential DNA Sequence Motif Identified (EDM1) & CpG%
(Susceptibility epigenetic transgenerational mark)

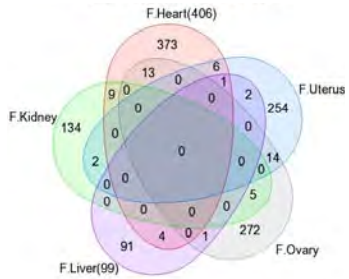
Genome Activity Alterations?
(transcriptome)

E16 Testis Transcriptome

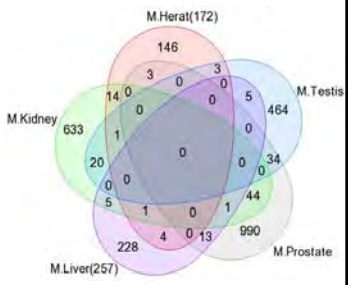


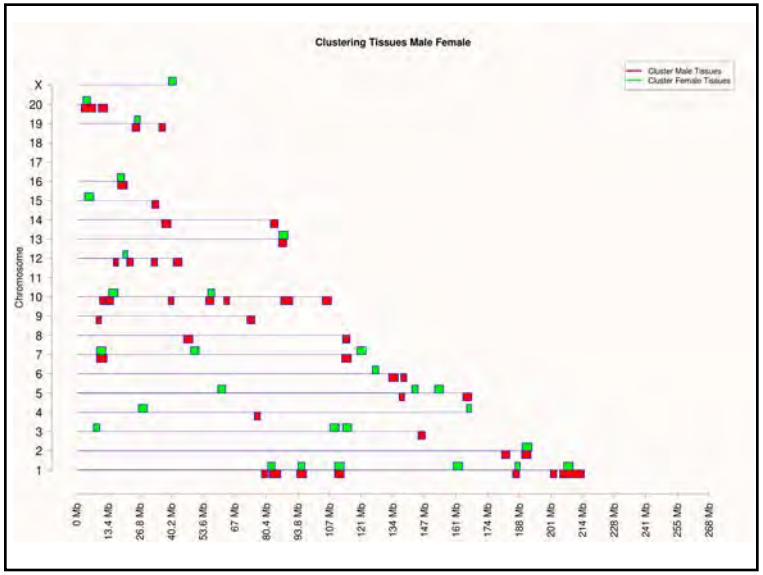
Tissue Specific Transgenerational Transcriptomes (F3)

Female

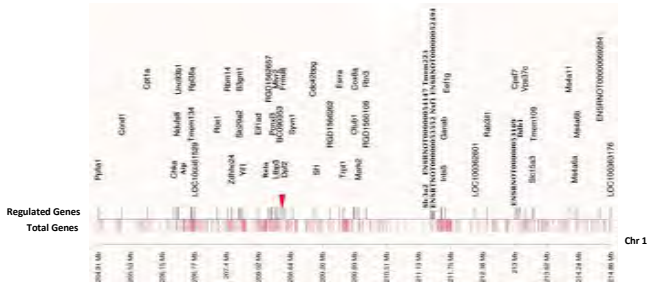


Male

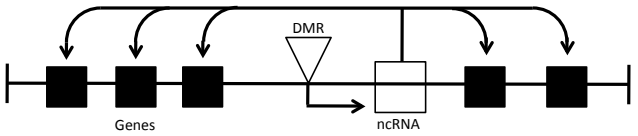




Epigenetic Control Regions



Epigenetic Control Region



Transgenerational Transcriptomes (F3 Generation)

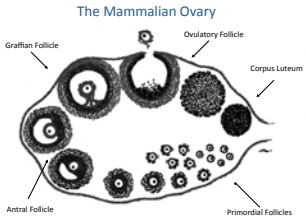
-Variety Somatic Tissues and Cell Types

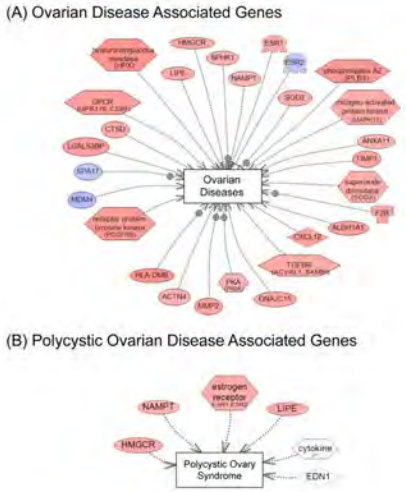
-Cell and Tissue Specific Altered Transcriptomes
(>10X more genes than epimutations)

-Gene Clusters associate with Epimutations
(Epigenetic Control Regions)

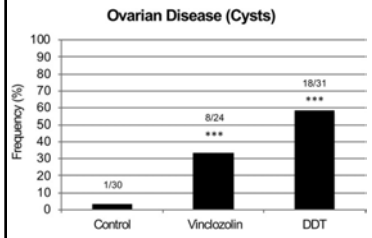
-Epimutation transmission to disease?

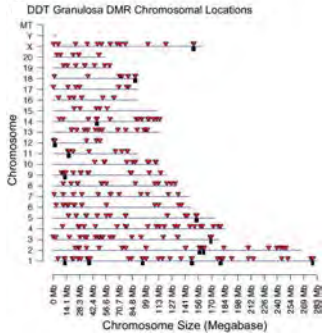
Ovarian
Granulosa Cell
Vinclozolin Lineage
F3 Generation
Transgenerational
Transcriptome
(~500 genes)



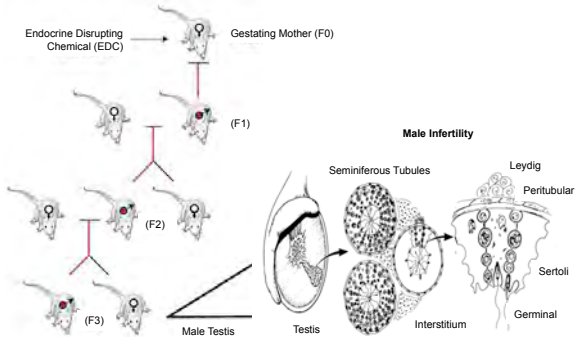


Environmental toxicant induced epigenetic transgenerational inheritance of ovarian pathology and granulosa cell epigenome and transcriptome alterations: ancestral origins of polycystic ovarian syndrome and primary ovarian insufiency.
Nilsson E, Klukovich R, Sadler-Riggleman I, Beck D, Xie Y, Yan W, Skinner MK.
Epigenetics. 2018;13(8):875-895.

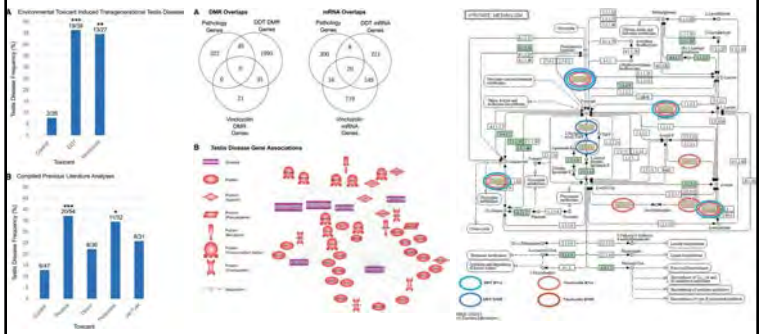




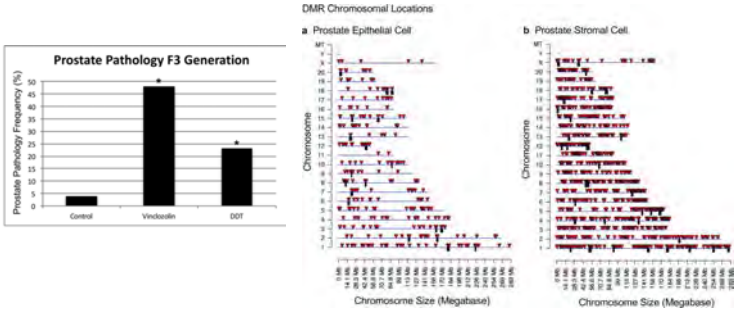
Epigenetic Transgenerational Inheritance of Sertoli Cell Abnormalities



Epigenetic transgenerational inheritance of testis pathology and Sertoli cell epimutations: generational origins of male infertility.
Sadler-Riggleman I, Klukovich R, Nilsson E, Beck D, Xie Y, Yan W, Skinner MK.
Environ Epigenet. 2019 Aug 29;5(3):dvz013.

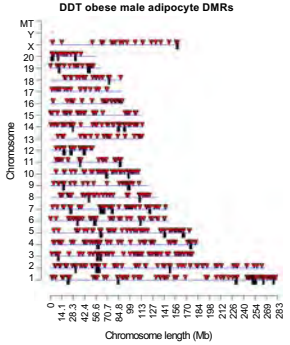


Environmental Toxicant Induced Epigenetic Transgenerational Inheritance of Prostate Pathology and Stromal-Epithelial Cell Epigenome and Transcriptome Alterations: Ancestral Origins of Prostate Disease.
Klukovich R, Nilsson E, Sadler-Riggleman I, Beck D, Xie Y, Yan W, Skinner MK.
Sci Rep. 2019 Feb 18;9(1):2209.

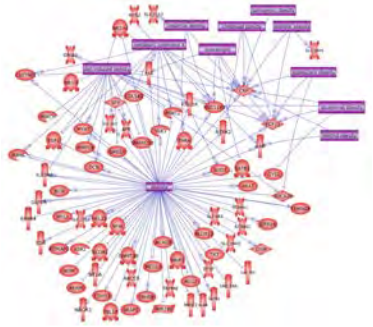


Adipocyte Epigenetic Alterations and Potential Therapeutic Targets in Transgenerationally Inherited Lean and Obese Phenotypes Following Ancestral Exposures
King SE, Nilsson E, Beck D, Skinner MK
Adipocyte 2019 8(1) 362-378

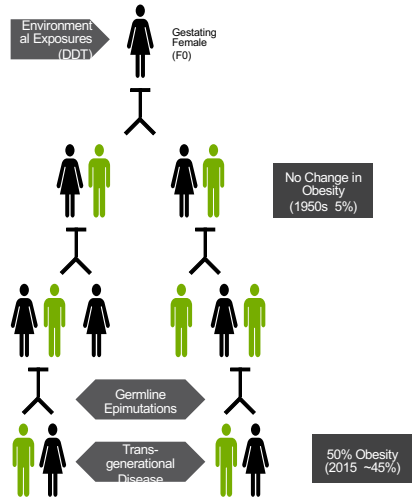
DDT obese male adipocyte DMRs



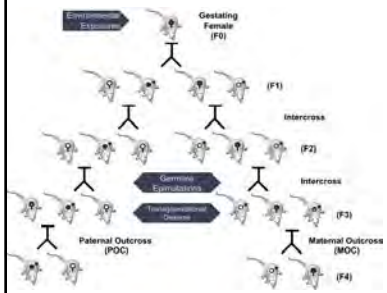
DMR Associated Genes Correlated with Obesity and Metabolic Disorders



EPIGENETIC TRANSGENERATIONAL INHERITANCE



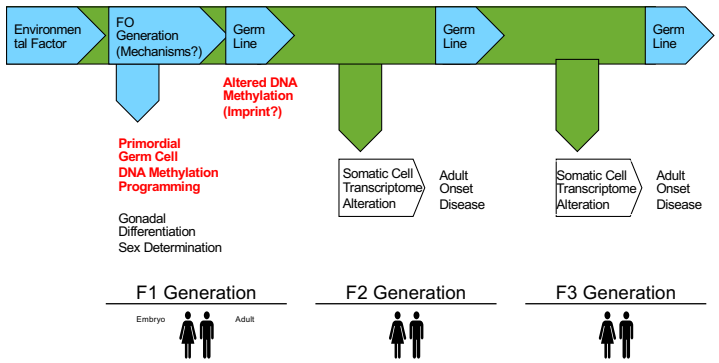
Epigenetic transgenerational inheritance of parent-of-origin allelic transmission of outcross pathology and sperm epimutations.
Ben Maamar M, King SE, Nilsson E, Beck D, Skinner MK.
Dev Biol. 2020 Feb 1;458(1):106-119.



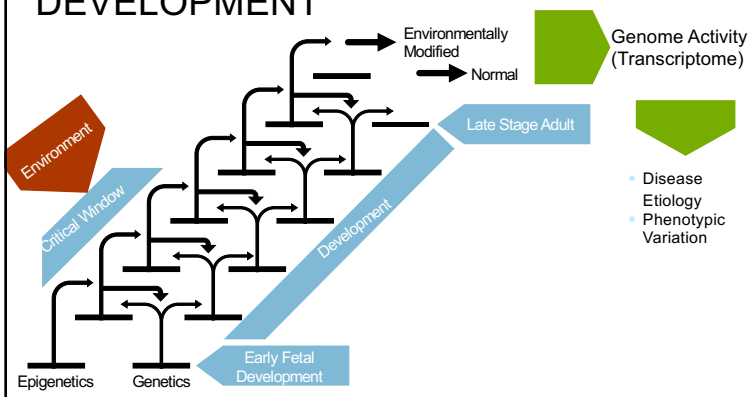
A Pathology parent-of origin-transmission

Diseases	F3 DDT	DDT F4 POC	DDT F4 MOC	DDT Allelic transmission	F3 Vinc	Vinc F4 POC	Vinc F4 MOC	Vinc Allelic transmission
Testis	+	-	-	Both alleles	+	-	-	Both allele
Ovary	+	-	-	Both alleles	-	-	-	No allele
Prostate	+	-	-	Both alleles	+	-	+	Maternal allele
Male Kidney	-	-	-	No allele	+	+	+	Either allele
Female Kidney	+	+	-	Paternal allele	-	+	-	Paternal allele
Male obesity	+	+	+	Either allele	-	+	+	Either allele
Female obesity	+	-	-	Both allele	+	+	-	Paternal allele
Male multiple diseases	+	-	+	Maternal allele	+	-	+	Maternal allele
Female multiple diseases	+	+	-	Paternal allele	+	+	-	Paternal allele
Female tumor	-	-	-	No allele	-	-	+	Maternal allele

ROLE OF GERM LINE IN EPIGENETIC TRANSGENERATIONAL INHERITANCE



EPIGENETIC AND GENETIC CASCADE OF EVENTS INVOLVED IN DEVELOPMENT



Summary

- Epigenetic Transgenerational Inheritance
  - -Non-Genetic Form Inheritance
  - -Toxicology / Environmental
  - -Molecular Mechanism for Disease Etiology and DOHAD
  - -All Epigenetic Processes Involved and Integrated in Germline
  - -Generational Toxicology

Environmental Epigenetics, Disease and Evolution
- -Integration Epigenetics and Genetics Essential Biology
- -Evolution and Disease Etiology Requires Inclusion Epigenetics
- -Doom and Gloom/ Biomarkers & Preventative Medicine

Epigenetic Alterations Promote Genetic Instability

Genetic Mutation	Epigenetic Alteration	DNA Sequence Alteration
Point Mutation (SNP)	DNA Methylation (CpG)	Susceptibility C➤T Conversion
Copy Number Variation (CNV)	Hypomethylation (Repeats)	Susceptibility Repeat Element Alteration (CNV)
Transposon Migration	Hypomethylation DNA	Susceptibility Transposon Migration
Translocation	DNA Methylation and Histone Alterations	Susceptibility Translocation at Break Point
Telomere Length	DNA Methylation Alteration	Alteration in Telomere Length

Environmentally Induced Epigenetic Transgenerational Inheritance of Sperm Epimutations Promote Genetic Mutations

Skinner MK, Guerrero-Bosagna C, Haque M.
Epigenetics 2015; 10:8, 762-771

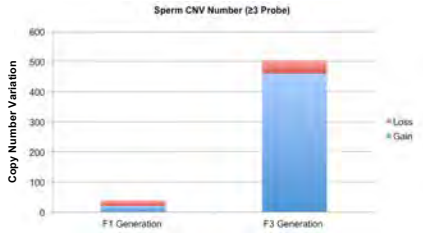
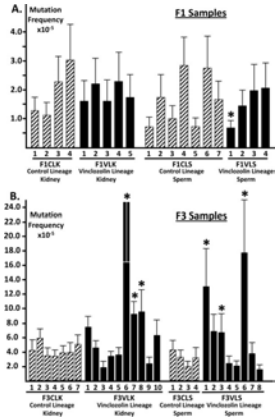


Table 1. (A) Vinclozolin F3 Generation Sperm Genome-wide CNV and Epimutations

Parameters	F1 Generation Sperm CNV	F3 Generation Sperm CNV	F3 Generation Epimutation Sperm
Number (Single Probe)	540(294 Gain / 246 Loss)	4912(4648 Gain / 264 Loss)	9932
Number (≥3 Probe)	39(21 Gain / 18 Loss)	506(461 Gain / 45 Loss)	191
Mean Size (base)	11,633	12,637	2,131
Mean CpG Density (CpG/100 bp)	1.1	1.0	0.9

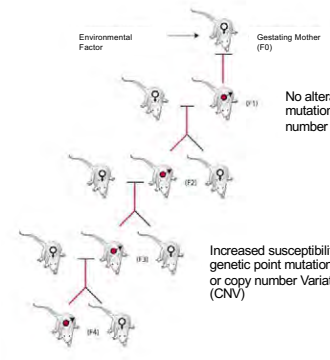
Tertiary Epimutations - A Novel Aspect of Epigenetic Transgenerational Inheritance Promoting Genome Instability
PLoS One. 2016 Dec 19;11(12):e0168038.
McCarrey JR, Lehle JD, Raju SS, Wang Y, Nilsson EE, Skinner MK.



Transgenerational Phenotype

	DNA Mutation	Epigenetic Mutation
Frequency -	<0.01% (Hot Spot 1-5%)	High (30-100%)
Reproducible-	Random Event	Highly Reproducible
Genetics-	Mendelian (decline frequency generationally)	Non-Mendelian

Sperm Epimutations Promotes Epigenetic Transgenerational Inheritance of Genetic Mutations



No alterations in genetic point mutations (SNP) or copy number Variation (CNV)

Increased susceptibility in genetic point mutations (SNP) or copy number Variation (CNV)

Epigenetics 2015 10:762
PlosONE 2016 e0168038

Summary

- Epigenetic Transgenerational Inheritance
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Evolutionary Biology

-Mechanism-Adaptation (ie DNA mutations)
-Problem- Timing
-Environment- Influence (?How)

-Determinant- Sexual Selection

Collaboration- David Crews, UT Austin

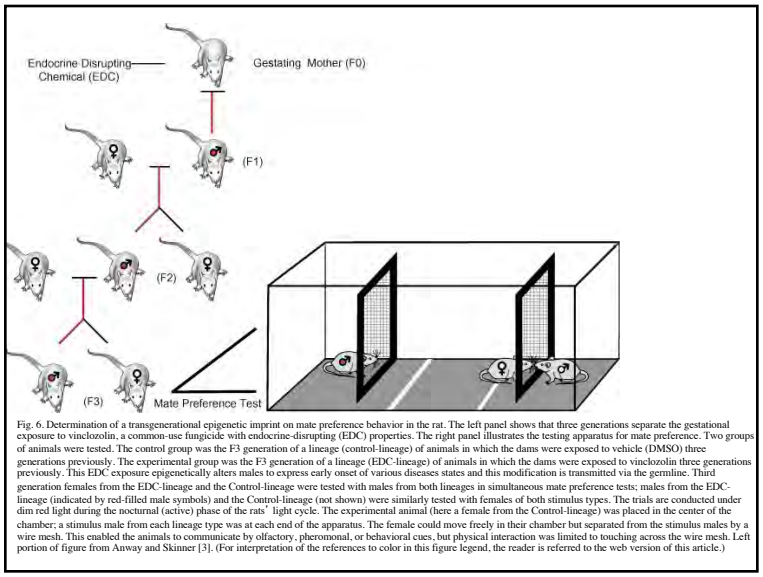
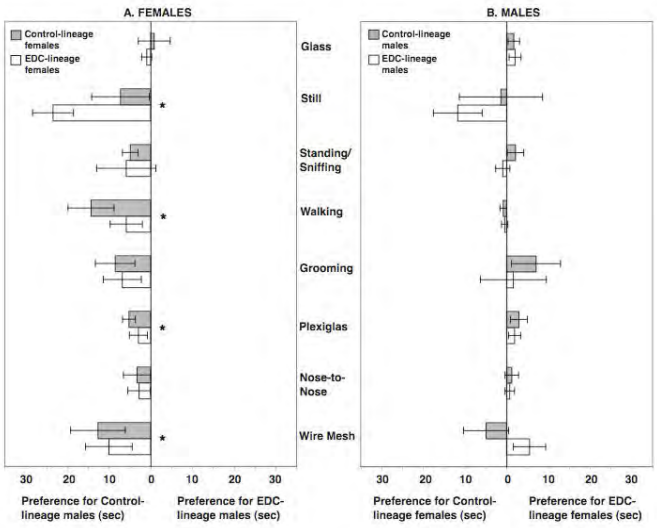
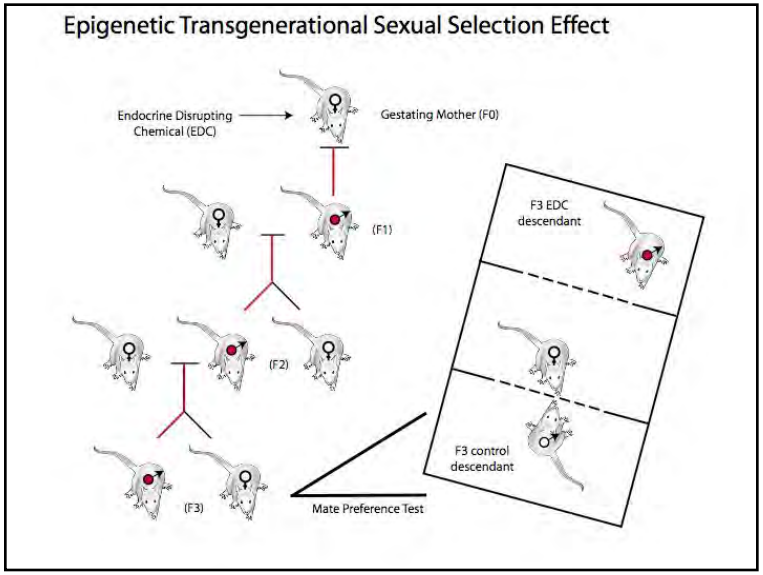


Fig. 6. Determination of a transgenerational epigenetic imprint on mate preference behavior in the rat. The left panel shows that three generations separate the gestational exposure to vinclozolin, a common-use fungicide with endocrine-disrupting (EDC) properties. The right panel illustrates the testing apparatus for mate preference. Two groups of animals were tested. The control group was the F3 generation of a lineage (control-lineage) of animals in which the dams were exposed to vehicle (DMSO) three generations previously. The experimental group was the F3 generation of a lineage (EDC-lineage) of animals in which the dams were exposed to vinclozolin three generations previously. This EDC exposure epigenetically alters males to express early onset of various diseases states and this modification is transmitted via the germline. Third generation females from the EDC-lineage and the Control-lineage were tested with males from both lineages in simultaneous mate preference tests; males from the EDC-lineage (indicated by red-filled male symbols) and the Control-lineage (not shown) were similarly tested with females of both stimulus types. The trials are conducted under dim red light during the nocturnal (active) phase of the rats' light cycle. The experimental animal (here a female from the Control-lineage) was placed in the center of the chamber; a stimulus male from each lineage type was at each end of the apparatus. The female could move freely in their chamber but separated from the stimulus males by a wire mesh. This enabled the animals to communicate by olfactory, pheromonal, or behavioral cues, but physical interaction was limited to touching across the wire mesh. Left portion of figure from Anway and Skinner [3]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)





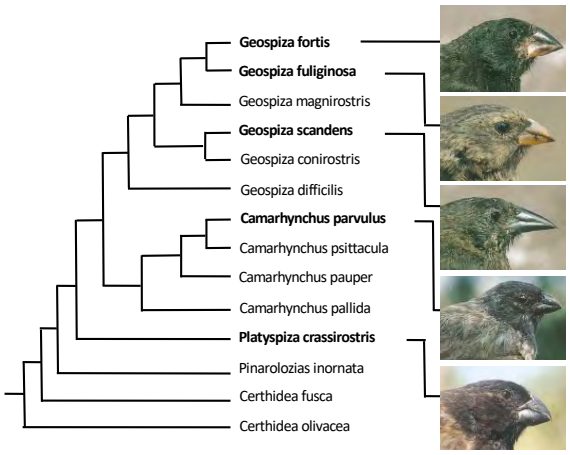
Role of Epigenetics in the Speciation of Darwin Finches and Evolutionary Biology

Goal-
Demonstrate the role of environment and epigenetics as critical molecular factors in evolutionary biology.

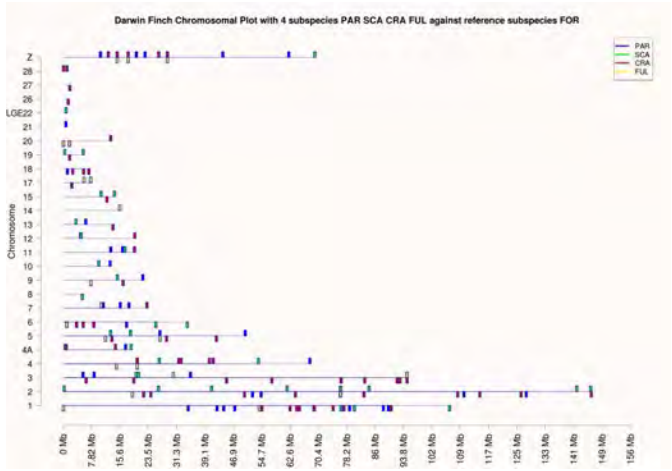
Objectives-
-Use Darwin Finches to determine the genetic and epigenetic differences between variety species (ie For, Ful, Spa, Par, Cra).

-Use Chromosomal Genomic Hybridization (CGH) to associate copy number variation between species (genetic differences).

-Use Methyated DNA Immunoprecipitation followed by tiling array (MeDIP-Chip) to associate epimutations between species (epigenetic differences).

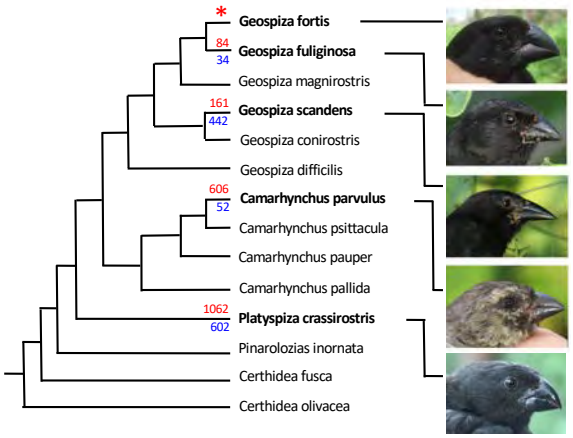


Speciation of Darwin Finch- Role Epigenetics and Genetics



Collaboration- Dale Clayton, Utah

Epigenetics and Darwin Finch Speciation and Evolution



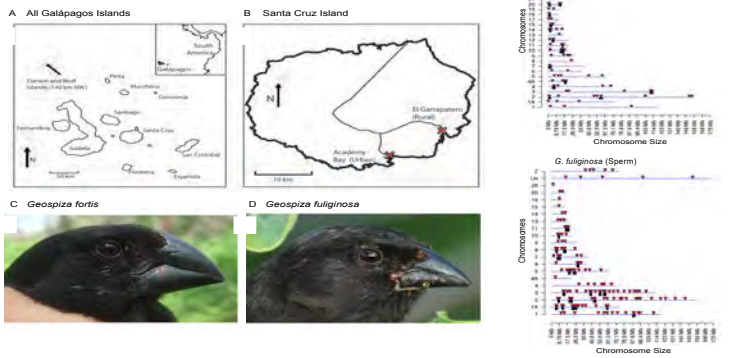
* – Reference Species
Red – Epimutations (DMR)
Blue – Genetic (CNV)

Collaboration- Dale Clayton Utah

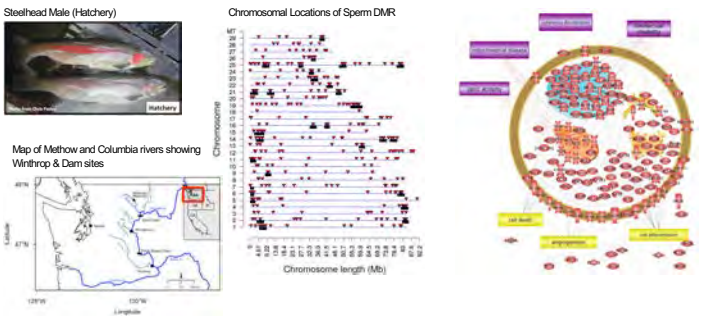
Epigenetic variation between urban and rural populations of Darwin's finches

Sabrina M. McNew[1], Daniel Beck[2], Ingrid Sadler-Riggleman[2], Sarah A. Knutie[1], Jennifer A. H. Koop[1], Dale H. Clayton[1] and Michael K. Skinner[*2]

BMC Evolutionary Biology (2017)



Differential DNA Methylation in Somatic and Sperm Cells of Hatchery vs Wild (natural origin) Steelhead Trout Populations (Environmental Epigenetics, 2021 1-17 dvab002), Nilsson E et al & Skinner MK



Role of environmentally induced epigenetic transgenerational inheritance in evolutionary biology: Unified Evolution Theory.
Skinner MK, Nilsson EE.
Environ Epigenet. 2021 Oct 30;7(1):dvab012.

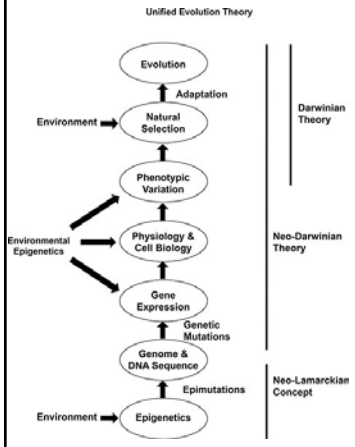
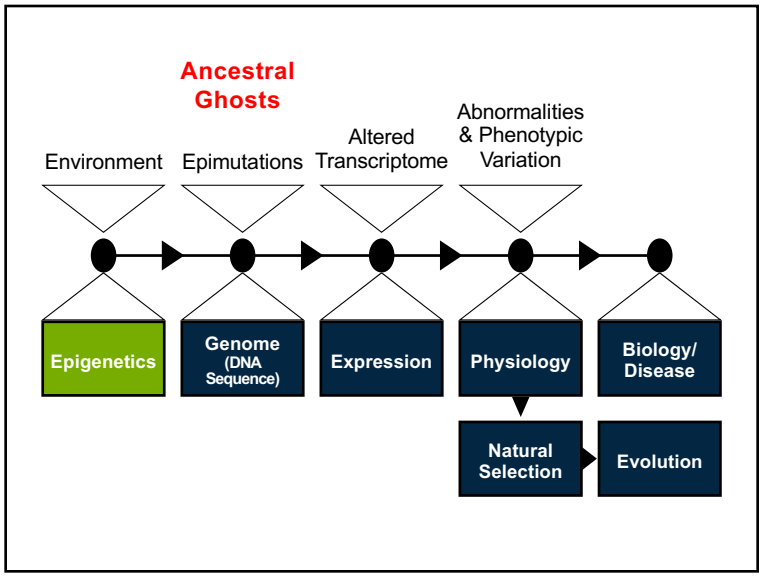


Table 5: Evolution theory components

Neo-Lamarckian Concept:	Environment directly alters phenotype generationally
Darwinian Evolution Theory:	Natural selection acts on phenotypic variation for adaptation
Neo-Darwinian Evolution Theory:	Genetic mutations promote phenotypic variation on which natural selection acts
Environmental Epigenetics:	Environmental epigenetic alterations promote phenotypic variation and facilitate genetic mutations to influence adaptation and natural selection
Unified Evolution Theory:	Environmental epigenetics and genetic mutations both promote heritable phenotypic variation on which natural selection acts



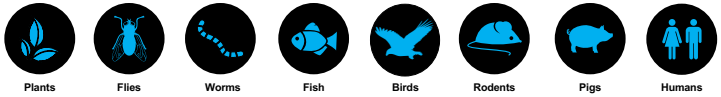
ENVIRONMENTALLY INDUCED EPIGENETIC TRANSGENERATIONAL INHERITANCE

Environmental Toxicants

Vinclozolin (Agricultural Fungicide)	Permethrin & DEET (Insect Repellants)
Methoxychlor (Agricultural Pesticide)	DDT (Pesticide)
Dioxin/TCDD (Industrial Contaminant)	Tributyltin (Industrial Toxicant & Biocide)
Plastic Compounds (BPA & Phthalates)	Hydrocarbons (Jet Fuel)
Atrazine (Herbicide)	Glyphosate (Pesticide / Herbicide)

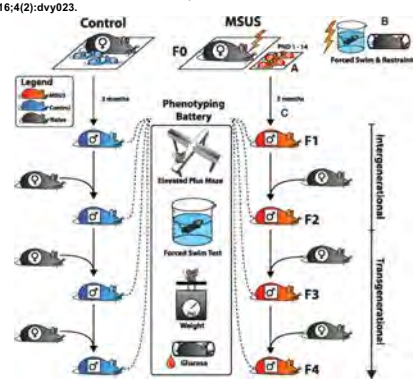
Other Types Exposures

Nutrition (High Fat or Caloric Restriction)	Smoking & Alcohol
Temperature & Drought (Plant Health & Flowering)	Stress Trauma (Behavioral)

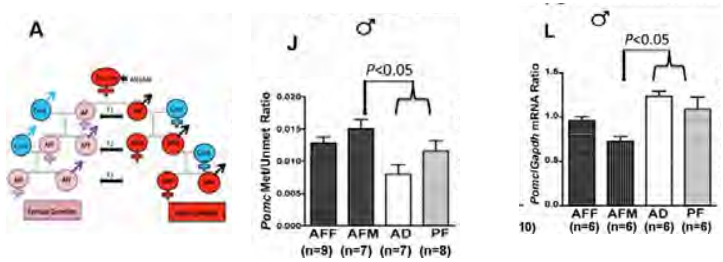


Exposure	Pathology	Reference
Toxicants		
Vinclozolin	Testis, Prostate, Kidney Disease, Tumors, Immune	Anway, et al., 2005 [3]; 2006 [32]
	Gender Specific Changes in Anxiety-like Behavior	Skinner, et al., 2008 [33]
	Immune and Reproductive	Nilsson, et al., 2008 [34]
Methoxychlor	Testis,	Anway, et al., 2005 [3],
	Kidney, Ovary, Obesity	Manikkam, 2014 [35]
Permethrin/DEET	Prostate, Kidney Disease	Manikkam, et al., 2012 [36]
Dioxin	Prostate, Kidney,	Manikkam, et al., 2012 [37]
	Fertility, Pregnancy	Bruner-Tran, 2011 [38]
BPA/Phthalates	Prostate, Kidney, Obesity	Manikkam et al., 2013 [39]
Hydrocarbon Mixture (Jet Fuel)	Prostate, Kidney, Obesity, Immune and Reproduction	Tracey et al., 2013 [40]
Vinclozolin, Permethrin/DEET, Plastics, Dioxin, Jet Fuel	Polycystic Ovaries, Reduced Primordial Follicle Pool	Nilsson et al., 2012 [41]
DDT	Obesity, Kidney, Testis	Skinner, et al., 2013 [5]
Phthalate	Testis and Spermatogonial Stem Cell	Doyle, et al., 2013 [42]
Tributyltin	Obesity and Adipose Cell	Chamorro-Garcia, et al., 2013 [43]
BPA	Social Behavior, Implantation, Litter Size, Sperm	Wolstenholme, et al., 2012 [44]; Salian, et al., 2009 [45]
Others		
Caloric Restriction	Cardiovascular Mortality	Bygren, et al., 2014 [46]
High Fat Diet	Growth and Insulin Sensitivity	Dunn and Bale, 2011 [6]
Folate	Congenital Malformations	Padmanabhan, et al., 2013 [47]
Drought	DNA Methylation Changes	Zheng, et al., 2013 [7]
Heat/Salt	Flowering and Salt Tolerance	Suter and Widmer, 2013 [48]
Prediabetes	Glucose Tolerance and Insulin Sensitivity	Wei, et al., 2014 [49]
Smoking	Abnormal Pulmonary Function	Rehan, 2013 [50]
Alcohol	Endocrine and Neuronal Function	Govorko, 2012 [51]
Heat Stress	Increased Hsp70 Production and Tolerance to Heat Stress	Norouzitallab, et al., 2014 [8]

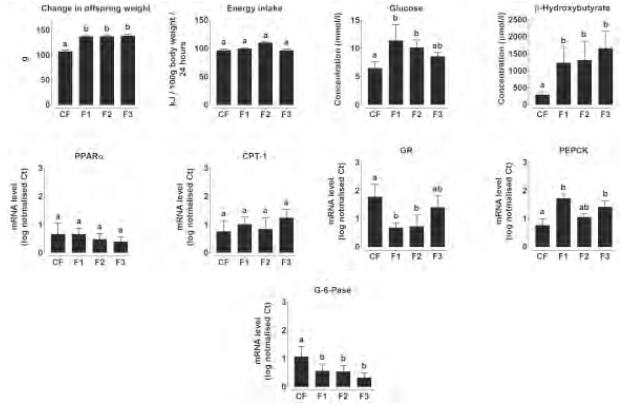
Transgenerational inheritance of behavioral and metabolic effects of paternal exposure to traumatic stress in early postnatal life: evidence in the 4th generation.
van Steenwyk G, Roszkowski M, Manuella F, Franklin TB, Mansuy IM.
Environ Epigenet. 2018 Oct 16;4(2):dvy023.



Male germline transmits fetal alcohol adverse effect on hypothalamic proopiomelanocortin gene across generations.
Govorko D, et al. (2012) Biol Psychiatry. 1;72(5):378-88.

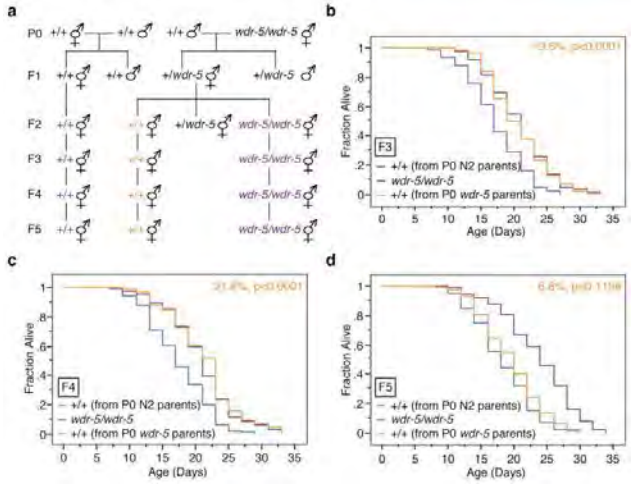


Progressive, transgenerational changes in offspring phenotype and epigenotype following nutritional transition.
Burdge GC, et al. (2011) PLoS One.6(11):e28282.



Offspring phenotype and mRNA expression of genes involved in hepatic gluconeogenesis and ketogenesis.
Change in offspring body weight on day 70 compared to weaning, offspring energy intake on day 70, fasting glucose and β-hydroxybutyrate concentrations on postnatal day 70. Hepatic PPARα, carnitine palmitoyltransferase-1 (CPT-1), glucocorticoid receptor (GR), phosphoenolpyruvate carboxykinase (PEPCK), (I) glucose-6-phosphatase (G-6-Pase) mRNA expression. Values are mean ± SD for n = 5–7 rats per group. Values with different letters are significantly different (P<0.05).

Transgenerational epigenetic inheritance of longevity in Caenorhabditis elegans.
Greer EL1, et al. (2011) Nature. 19;479(7373):365-71.

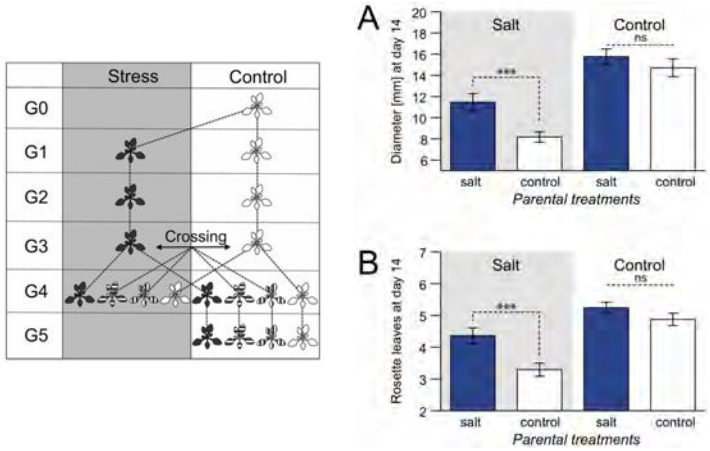


Transgenerational epigenetic inheritance in plants.
Hauser MT, et al. (2011) Biochim Biophys Acta. 2011 Aug;1809(8):459-68.

Table 1
Impact of variations in the epigenetic status on plant growth and development.

Plant species	Induction	Epigenetic status	Effect	Reference
Oryza sativa DWARF1		DNA hypomethylation	Growth	[209,210]
Wheat *Triticum aestivum*	Glutenin gene	DNA methylation		[211]
Triticale		Hypermethylation	Induced growth	[212]
Tobacco *Nicotiana tabacum*	Transgenesis	Loss of expression of transgene, hypermethylation of T-DNA.	Transgene silencing	[213]
Arabidopsis	Transgenesis	Loss of expression of *hpt* transgene	Hygromycin sensitivity	[214]
Petunia	Transgenesis	Loss of expression of transgene, hypermethylation of T-DNA		[215]
Arabidopsis AGAMOUS	*met1, ddm1* background	DNA hypermethylation	Floral organ development, patterning	[216,217]
Silene *latifolia*	5-azacytidine	DNA hypomethylation	Sex change to androhermaphrodisism	[218]
Arabidopsis SUPERMAN/clark kent	*met1, ddm1* background	DNA hypermethylation	Floral organ development, patterning	[219,220]
Arabidopsis PAI (phosphoribosylanthranilate isomerase)	Natural	Ecotypes with hypermethylated multiplied *PAI* genes, result in the silencing of the unlinked PAI genes	PAI2 silencing results in blue fluorescing plants	[221–224]
Linaria vulgaris LCYCLOIDEA	Natural	Cytosine DNA methylation of glutenin genes	Floral development	[36]
Arabidopsis FLOWERING WAGENINGEN (FWA)	Natural	Loss of methylation, ectopic expression of FWA	Late flowering	[225]
Arabidopsis *MAGPRI* (constitutive expressor of PR genes 1)	*ddm1* background	Overexpression of CPR1	Twisted, leaves, dwarfing and reduced fertility	[226]
Arabidopsis Flowering Repressor Locus (FLC)	Natural	H3K9 and H3K27 dimethylation at FLC locus after vernalization	Flower induction after vernalization	[227,228]
Flax *Linum usitatissimum*	5-azacytidine	DNA hypomethylation, chromatin remodeling	Number of leaves, flowering age, stem height	[229,230]
Flax *Linum usitatissimum*	Nutrient conditions, heat	DNA methylation; presence of insertion element	Biomass, growth	[136]
Tomato	Natural	SBP-Box gene	Fruit ripening	[231]
Arabidopsis	Natural	Non-LTR retrotransposon Sadhu is differentially expressed in natural accessions as Col (active), Cvi and Ler (inactive)		[232]
Arabidopsis BONSAI	*ddm1* background	DNA hypermethylation and silencing of APC13/BNS gene by nearby a long interspersed	Growth	[233]
Melon *Cucumis myriocarpus*	Natural transposon insertion	Spreading of DNA methylation to WIP1 promoter	Sex determination, expression of WIP1 leads to carpel abortion	[234]
Wild potato *Solanum raiz-lealii* Brüch.	Species of hybrid origin	Phenotypic instability of flower development	Flower development	[235]

Environmental heat and salt stress induce transgenerational phenotypic changes in Arabidopsis thaliana.
Suter L, Widmer A. (2013) PLoS One. 9;8(4):e60364.



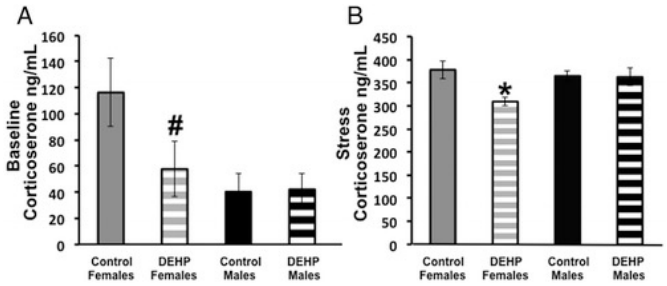
Exposure to the environmental endocrine disruptor TCDD and human reproductive dysfunction: Translating lessons from murine models
Reprod Toxicol. 2017 Mar;68:59-71.
Bruner-Tran KL, Gnecco J, Ding T, Glore DR, Pensabene V, Osteen KG.

Cumulative overview of reproductive characteristics in control and toxicant exposed female mice.[a]

Mouse History	Pregnancy Rate	Term Delivery[b]	Adenomyosis	Methylation of PR[c]
Control	95–100%	100%	0%	0%
F1	46	37	70	66
F3	50	60	56	33

[a] Data compiled from references [10,101,102].
[b] Delivery was considered term when occurring E19-E21 (plug date is considered E0.5).
[c] Whole uteri collected from non-pregnant mice at estrus.

Transgenerational Effects of Di-(2-Ethylhexyl) Phthalate (DEHP) on Stress Hormones and Behavior.
Quinnies KM, Doyle TJ, Kim KH, Rissman EF.
Endocrinology. 2015 Sep;156(9):3077-83.



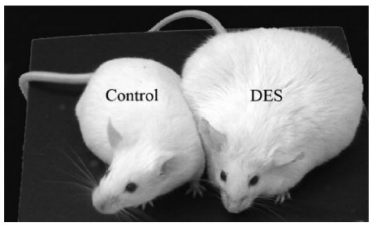
Mean (±SEM) corticosterone levels (ng/mL) measured in plasma from 150-mg/kg DEHP-female and control F3 mice.

(A) Under baseline conditions DEHP-lineage mice tended to have lower corticosterone levels than control females (#P = .10). (B) After 15 minutes of restraint stress, DEHP-lineage females had lower corticosterone than control females; *, P < .05 (control-lineage females stressed, n = 10; control-lineage females baseline, n = 9; DEHP-lineage females stressed, n = 8; DEHP-lineage females baseline, n = 10; control-lineage males stressed, n = 6; control-lineage males baseline, n = 6; DEHP-lineage males stressed, n = 8; DEHP-lineage males baseline, n = 7).

The history of Distilbène® (Diethylstilbestrol) told to grandchildren--the transgenerational effect.
Fénichel P, Brucker-Davis F, Chevalier N.
Ann Endocrinol (Paris). 2015 Jul;76(3):253-9.

Table 1
In utero exposure to Distilbène®: historical.

1936	DES Synthesis. Dodd and Larson. [22]
1946	Distilbène® advocated for spontaneous miscarriage and premature labour: 5 to 125 mg/day from 6WA to 35WA
1953	Randomised double-blind study $n = 840$ not superior to placebo. Dieckman. [3]
1970	7 cases of clear cell adenocarcinoma (CCA) of the vagina and cervix in very young women exposed to DES in uteru. Herbst [4]
1972	US ban by the FDA
1975	First case of CCA described in France
1976	Disappears from medical references as treatment for miscarriage in France
1977	Excluded for pregnancy
1977	Uterine defects related to DES. Kauffmann. [23]
1981	Pregnancy complications. Papiernik. [24]
1990	Genital abnormalities described in men
2006	RR × 3 breast cancer after 50 years of age in second generation. Palmer. [1]
2002	Third generation hypospadias. Klip. [2]
2009	Epigenetic mechanisms of foetal programming after exposure to DES hypermethylation of HOX 10 gene in uterine cancer. Bromer. [25]



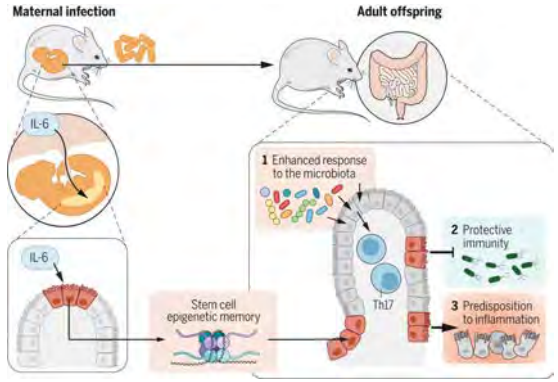
Genital tract and reproductive characteristics in daughters of women and men prenatally exposed to diethylstilbestrol (DES)
Wautier A, Tournaire M, Devouche E, et al.
Therapie. Sep-Oct 2020;75(5):439-448.

Table 2 Uterine defects.

Uterine defects N %	Third generation women					General population		Second generation = prenatally exposed women
	Our population				Kaufman [25]	Nahum [30]		Kaufman [3]
	Total (a)	Exposed father (b)	Exposed father and mother (c)	Exposed mother (d)	Exposed mother (e)	Fertile (f)	Infertile (g)	Prenatally exposed women (h)
Population (aged 18 or older)	759 (380)	76 (34)	11 (7)	672 (339)	28	–	–	60
MRKH syndrome	3 0.4% (0.8%)	11.3% (2.9%)	0	2 0.3% (0.6%)	0	–	1/4500	–
Doubling uterus	3 0.4% (0.8%)	0	0	3 0.4% (0.9%)	0	–	1.2%	–
Bicornuate uterus	6 0.8% (1.6%)	0	0	6 0.9% (1.8%)	0	–	1.7%	–
All uterine defects	121.6% (3.2%)	11.3% (2.9%)	0	111.6% (3.2%)	0	0.17%	3.5%	40 66%

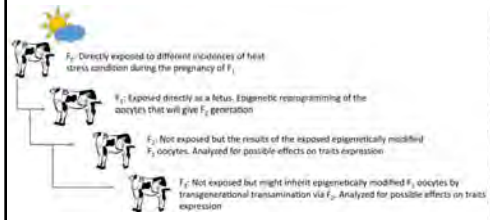
This table shows uterine defects in: third generation women = daughters of parents prenatally exposed to DES in our population (a,b,c,d), daughters of exposed mother in Kaufman et al., 2002 (e), general population (f, g), second generation = women prenatally exposed to DES (h), MRKH: Mayer-Rokitanski-Kuster-Hauser Syndrome.

Prenatal maternal infection promotes tissue-specific immunity and inflammation in offspring
Lim AI, McFadden T, Link VM, et al.
Science. 2021 Aug 27;373(6558):eabf3002.



Maternal infection promotes offspring intestine-specific immunity and inflammation.
The direct response of fetal intestinal epithelial cells to IL-6 during maternal infection confers an enduring epigenetic memory to adult intestinal epithelial stem cells. As such, offspring epithelial cells exhibit enhanced reactivity toward the microbiota and heightened ability to control oral infection. However, these responses come at the cost of greater predisposition to gut inflammation.

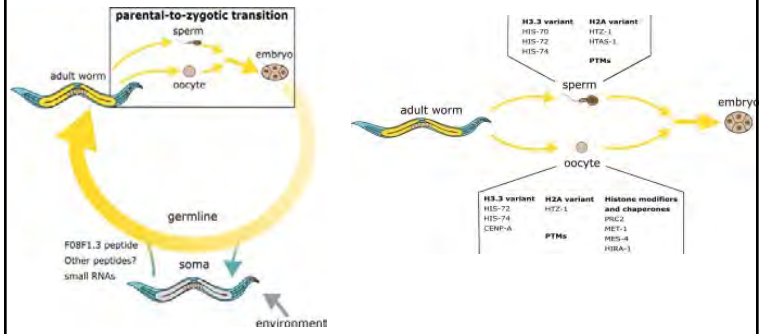
Broad phenotypic impact of the effects of transgenerational heat stress in dairy cattle: a study of four consecutive generations.
Weller JI, Ezra E, Gershoni M.
Genet Sel Evol. 2021 Sep 6;53(1):69.



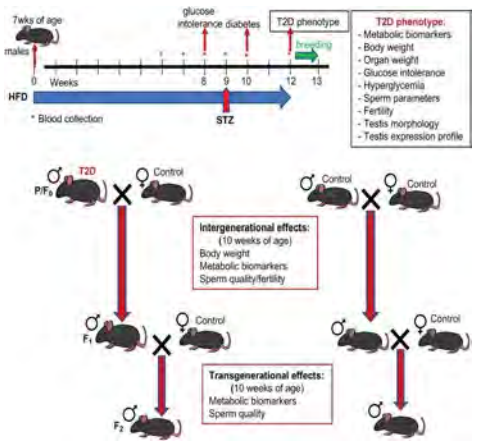
Trait	Root mean square error[a]	Probability	
		Dam	MGD
PD16	16.3	10^{-4*}	10^{-4*}
Milk	166	10^{-4*}	0.0248
Fat	8.57	10^{-4*}	10^{-4*}
Protein	4.98	10^{-4*}	10^{-4*}
Fat %	0.072	10^{-4*}	0.0031*
Protein %	0.079	0.0017*	NS[b]
SCS	0.126	10^{-4*}	0.0017*
Female fertility	1.38	NS	NS
Persistency	1.77	0.0341	NS
Herd life	37.5	0.0262	NS
Dystocia	0.972	10^{-4*}	10^{-4*}
Stillbirth	0.645	10^{-4*}	10^{-4*}
Maturing rate	0.0715	10^{-4*}	10^{-4*}

[a] Significant after Bonferroni's adjustment ($P = 0.005$); the Bonferroni correction was computed based on the 13 traits analyzed
* Derived from the dam analyses
[b] Not significant

Transmission of chromatin states across generations in C. elegans.
Özdemir I, Steiner FA.
Semin Cell Dev Biol. 2021 Nov 22;S1084-9521(21)00284-6.

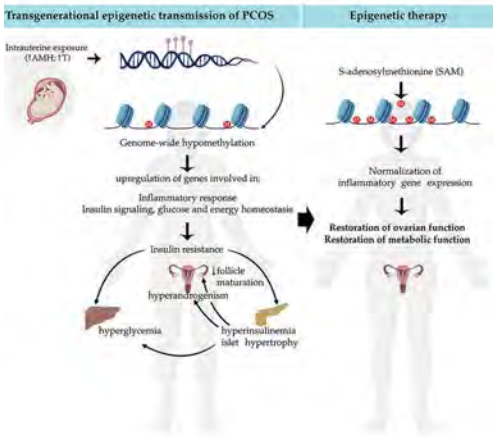


The Transgenerational Transmission of the Paternal Type 2 Diabetes-Induced Subfertility Phenotype
Zatecka E, Bohuslavova R, Valaskova F, et al.
Front Endocrinol (Lausanne). 2021 Nov 5;12:763863.



Experimental schematics. Type 2 diabetes (T2D) was induced by a combination of high fat diet (HFD) and a low dose of streptozotocin (STZ): sexually matured males (7 weeks old) were kept on HFD for 12 weeks before mating and injected with STZ at week 9 of the HFD experiment. T2D males and standard diet (control) fed males (parental generation, P/F_0) were mated with standard diet fed females. After one week mating pairs were separated, males were killed, and tissues were collected for further analyses. The F_1 male offspring were mated at 9 weeks of age, after one week mating pairs were separated. F_1 males were killed, and analyses were performed at 10 weeks of age. Effects of diabetic paternal environment was also evaluated in the second (F_2) male offspring generation at 10 weeks of age. Both F_1 and F_2 males were maintained on standard diet.

Polycystic ovary syndrome is transmitted via a transgenerational epigenetic process.
Mimouni NEH, Paiva I, Barbotin A-L, et al.
Cell Metab. 2021 Mar 2;33(3):513-530.e8.

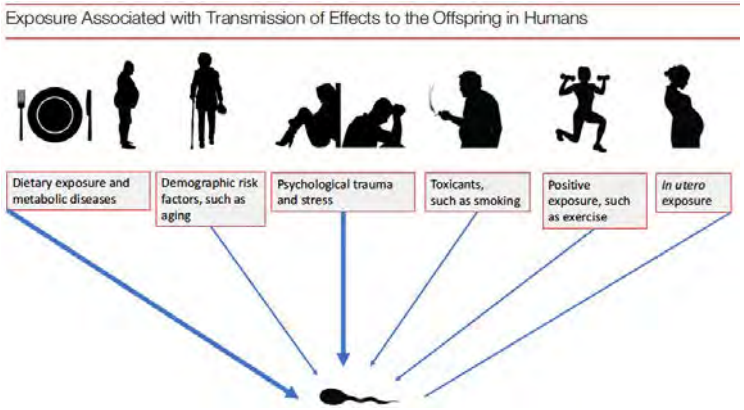


Impact of Parental Exposure on Offspring Health in Humans.
Jawaid A, Jehle K-L, Mansuy IM.
Trends Genet. 2021 Apr;37(4):373-388.

Table 6. *In Utero* Exposure

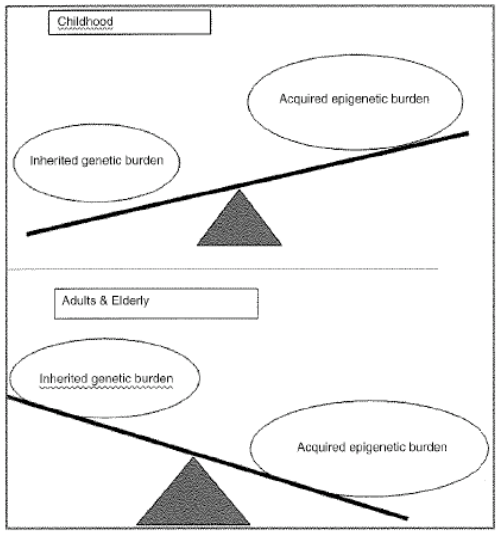
Type of disorder	Environmental exposure	Transmitted intergenerational/ transgenerational phenotype	Associated epigenetic changes	Number of affected offspring generations	Number of tested persons (*n*)	Refs
Nutritional/metabolic disorders	Changes in food availability in women	Increased risk of sudden death and cardiovascular mortality	Not assessed	2	277 families	[83]
	Maternal LBW[a] and IUGR[b]	Increased risk of LBW and IUGR in offspring	Not assessed	2	280 030 maternal observations 125 078 paternal observations	[84]
Toxicants	Maternal and grandmaternal smoking during pregnancy	Asthma	Not assessed	2	4082 offspring	[56]
	Grandmaternal smoking during pregnancy	Asthma (independent of mother's smoking status)	Not assessed	2	53 169 offspring	[36]
	Grandmaternal smoking during pregnancy	Asthma within the first 6 years of life (persistent childhood asthma phenotype)	Not assessed	2	44 583 grandmothers 46 197 mothers 65 966 offspring	[57]
	Grandmaternal smoking during pregnancy	Increased birth weight, birth length, and body mass index of grandsons	Not assessed	2	12 707 parents 9677 children	[58]

[a]LBW, low birth weight.
[b]IUGR, intrauterine growth retardation.



Fetal Basis of Adult Onset Disease

Embryo	Most Sensitive
Postnatal	Sensitive
Pubertal	Less Sensitive
Adult	Insensitive
Aged Adult	Most Disease

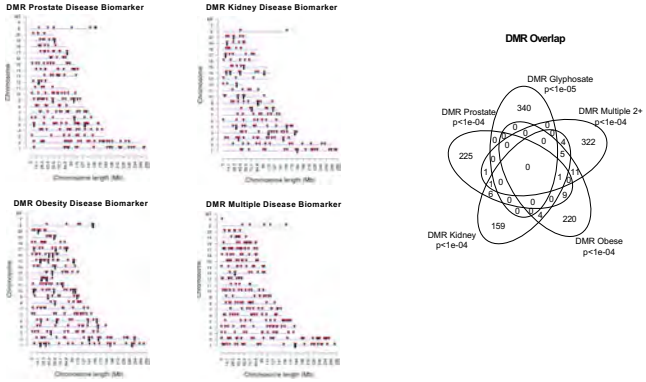


Summary

- Epigenetic Transgenerational Inheritance
  - -Non-Genetic Form Inheritance
  - -Toxicology / Environmental
  - -Molecular Mechanism for Disease Etiology and DOHAD
  - -All Epigenetic Processes Involved and Integrated in Germline
  - -Generational Toxicology

Environmental Epigenetics, Disease and Evolution
- -Integration Epigenetics and Genetics Essential Biology
- -Evolution and Disease Etiology Requires Inclusion Epigenetics
- -Doom and Gloom/ Biomarkers & Preventative Medicine

Glyphosate Induced Transgenerational DNA Methylation and Histone Retention Sperm Epigenetic Biomarkers for Disease
Ben Maamar M, Beck D, Thorson JLM, Nilsson E, Kubsad D, Skinner MK (2020) Epigenetics 9: 1-18



Environmental Induced Epigenetic Transgenerational Inheritance of Pathology: Systems Epigenetics in Disease Etiology and Generational Toxicology

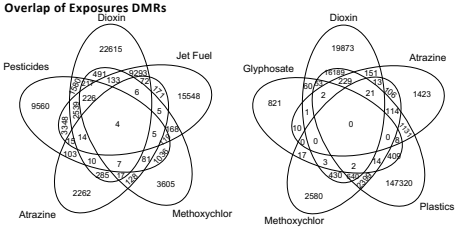
Daniel Beck, Eric E. Nilsson, Millissia Ben Maamar, Michael K. Skinner (Sci Reports 2022, 12:5452)

Exposure Specific DMRs

Exposure Induced F3 Generation Sperm DMRs at edgeR p-value p<1e-06

Jet fuel	Pesticides	Plastics	Dioxin	Methoxy	Glyphosate	Atrazine
p<1e-06	p<1e-06	p<1e-06	p<1e-06	p<1e-06	p<1e-04	p<1e-06
31,517	18,791	168,543	37,475	6188	1395	3166

Overlap of Exposures DMRs

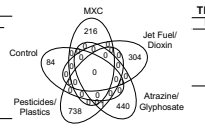


TRANSGENERATIONAL EXPOSURE DMRs ARE DISTINCT

Transgenerational Disease Specific DMRs are Distinct

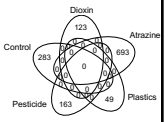
KIDNEY DISEASE

Exposure	p-value	All Window
Control	p<1e-04	84
Dioxin	p<1e-04	130
Pesticide	p<1e-04	463
Plastic	p<1e-04	276
Glyphosate	p<1e-04	180
Methoxychlor	p<1e-04	216
Atrazine	p<1e-04	261
Jet Fuel	p<1e-04	174



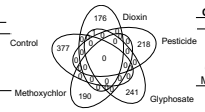
TESTIS DISEASE

Exposure	p-value	All Window
Control	p<1e-04	283
Dioxin	p<1e-04	123
Pesticide	p<1e-04	163
Plastic	p<1e-04	49
Atrazine	p<1e-04	693



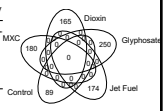
PROSTATE DISEASE

Exposure	p-value	All Window
Control	p<1e-04	377
Dioxin	p<1e-04	177
Glyphosate	p<1e-04	242
Methoxychlor	p<1e-04	190
Pesticide	p<1e-04	218



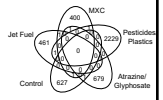
OBESITY

Exposure	p-value	All Window
Control	p<1e-04	89
Jet Fuel	p<1e-04	174
Dioxin	p<1e-04	165
Glyphosate	p<1e-04	250
Methoxychlor	p<1e-04	180



MULTIPLE PATHOLOGY

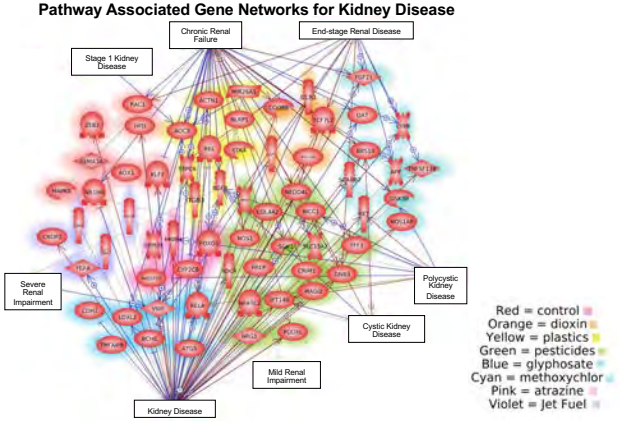
Exposure	p-value	All Window
Control	p<1e-04	629
Pesticide	p<1e-04	2100
Plastic	p<1e-04	131
Glyphosate	p<1e-04	345
Methoxychlor	p<1e-04	404
Atrazine	p<1e-04	336
Jet Fuel	p<1e-04	463



PUBERTY PATHOLOGY

Exposure	p-value	All Window
Atrazine	p<1e-04	322
Jet Fuel	p<1e-04	280

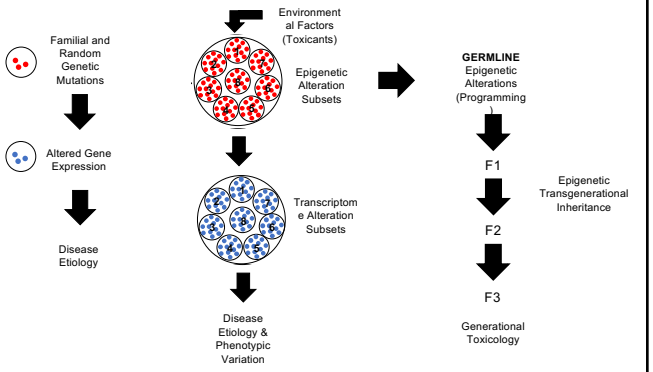
Pathway Associated Gene Networks for Kidney Disease



Novel Systems Epigenetics Disease Etiology Paradigm

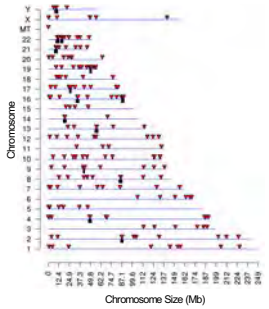
Genetic Determinism Disease Etiology Paradigm

Environmental Epigenetics and Genetic Disease Etiology Paradigm

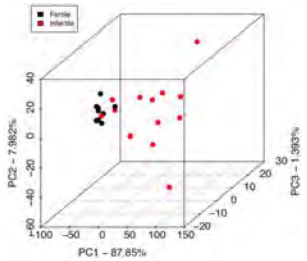


Sperm DNA Methylation Epimutation Biomarkers for Male Infertility and FSH Therapeutic Responsiveness
Luján S, Caroppo E, Niederberger C, Arce J-C, Sadler-Riggleman I, Beck D, Nilsson E, Skinner MK
Scientific Reports (2019) 9:16786

Infertility Sperm DMR Chromosomal Locations



PCA Infertility DMR Signature Analysis



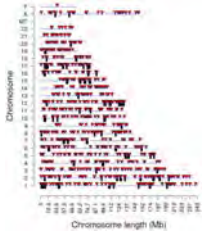
Sperm DNA Methylation Epimutation Biomarker for Paternal Offspring Autism Susceptibility
Garrido N, et al., and Skinner MK
Clinical Epigenetics 2021 (13:6)

DMR Identification

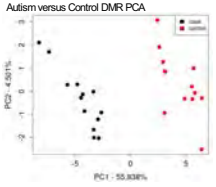
p-value	All Window	Multiple Window
0.001	13271	256
1e-04	3214	31
1e-05	**805**	**6**
1e-06	223	1
1e-07	60	0

Significant windows	1	2	3
Number of DMR	799	3	3

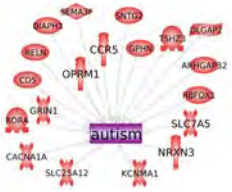
Chromosome Plot



Autism versus Control DMR PCA



DMR Associated Genes and Autism



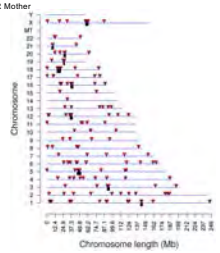
Preterm Birth Buccal Cell Epigenetic Biomarkers to Facilitate Preventative Medicine
Paul Winchester, Eric Nilsson, Daniel Beck, and Michael K. Skinner
(In Review)

Mother DMRs

p-value	All Window	Multiple Window
0.001	601	53
1e-04	**165**	**28**
1e-05	56	17
1e-06	32	12
1e-07	20	9

Significant windows	1	2	3	4	≥ 5
Number of DMR (p<1e-04)	137	18	5	2	3

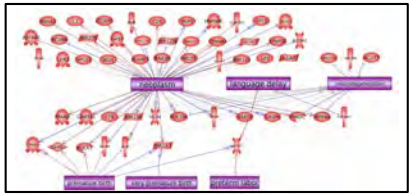
DMR Mother

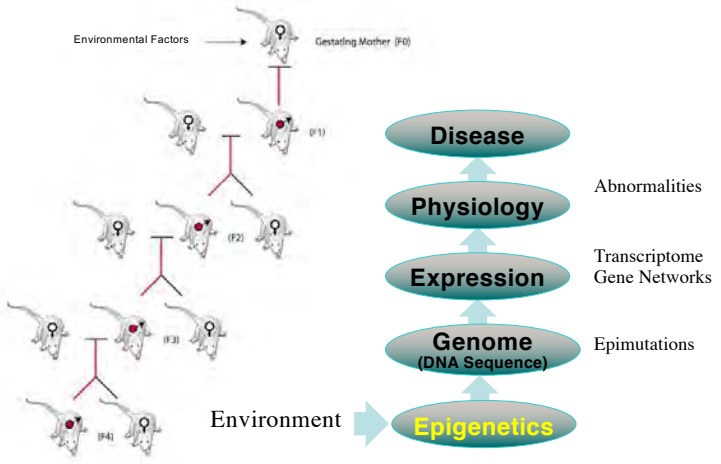


Extended Overlaps

p<1e-04 \ p<0.05	Mother	Father	Female Child	Male Child
Mother	165 (100%)	81 (49%)	96 (58%)	50 (30%)
Father	54 (74%)	73 (100%)	47 (64%)	34 (47%)
Female Child	79 (58%)	62 (46%)	136 (100%)	46 (34%)
Male Child	17 (28%)	15 (25%)	11 (18%)	81 (100%)

Mother DMR Associated Gene Correlations





"Systems Biology of Reproduction"

Spring 2022 (Even Years) – Course Syllabus
BIOL 475/575 Level Undergraduate/Graduate (3 Credit)
SLN: (475) – 05504, (575) – 05505
Time - Tuesday and Thursday 10:35 am-11:50 am
Course Lectures in person and and on Canvas/Panopto and Discussion Sessions in person and on WSU Zoom for all campuses
Room – CUE 418
Course Director – Michael Skinner, Abelson Hall 507, 335-1524, skinner@wsu.edu
Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, nilsson@wsu.edu
Learning Objective -
Current literature based course on the Systems Biology of Reproduction. Learning Systems approaches to the biology of reproduction from a molecular to physiological level of understanding.
Schedule/Lecture Outline –

January	11 & 13	Week 1	Systems Biology Introduction
	18 & 20	Week 2	Molecular/ Cellular/ Reproduction Systems
	25 & 27	Week 3	Sex Determination Systems
February	1 & 3	Week 4	Male Reproductive Tract Development & Function
	8 & 10	Week 5	Female Reproductive Tract Development & Function
	15 & 17	Week 6	Gonadal Developmental Systems Biology
	22 & 24	Week 7	Testis Systems Biology
March	1 & 3	Week 8	Ovary Systems Biology
	8 & 10	Week 9	Epigenetics and Transgenerational Gonadal Disease
	14 – 18	**Week 10**	**Spring Break**
	22 & 24	Week 11	Gametogenesis/ Stem Cells/ Cloning
	29 & 31	Week 12	Hypothalamus-Pituitary Development & Function
April	5 & 7	Week 13	Reproductive Endocrinology Systems
	12 & 14	Week 14	Fertilization & Implantation Systems
	19 & 21	Week 15	Fetal Development & Birth Systems
	26 & 28	Week 16	Assisted Reproduction/Contraception
May	3 & 5	Week 17	Exam or Grant Review