Folic acid supplementation reduces multigenerational sperm miRNA perturbation induced by in utero environmental contaminant exposure

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Abstract

Persistent organic pollutants (POPs) can induce epigenetic changes in the paternal germline. Here, we report that folic acid (FA) supplementation mitigates sperm miRNA profiles transgenerationally following in utero paternal exposure to POPs in a rat model. Pregnant founder dams were exposed to an environmentally relevant POPs mixture (or corn oil) ± FA supplementation and subsequent F1–F4 male descendants were not exposed to POPs and were fed the FA control diet. Sperm miRNA profiles of intergenerational (F1, F2) and transgenerational (F3, F4) lineages were investigated using miRNA deep sequencing. Across the F1–F4 generations, sperm miRNA profiles were less perturbed with POPs + FA compared to sperm from descendants of dams treated with POPs alone. POPs exposure consistently led to alteration of three sperm miRNAs across two generations, and similarly one sperm miRNA due to POPs + FA; which was in common with one POPs intergenerationally altered sperm miRNA. The sperm miRNAs that were affected by POPs alone are known to target genes involved in mammary gland and embryonic organ development in F1, sex differentiation and reproductive system development in F2 and

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cognition and brain development in F3. When the POPs treatment was combined with FA supplementation, however, these same miRNA-targeted gene pathways were perturbed to a lesser extend and only in F1 sperm. These findings suggest that FA partially mitigates the effect of POPs on paternally derived miRNA in an intergenerational manner.

Key words: organochlorine; transgenerational epigenetic inheritance; microRNA; prenatal exposure; folate

Introduction

Environmental pollutants, including persistent organic pollutants (POPs), pose ongoing threats to global ecosystems [1]. POPs are synthetic organic compounds that resist environmental degradation and are distributed via long-distance atmospheric transport mechanisms to deposit in colder regions, notably the Arctic [2, 3]. International restrictions have decreased POPs over the past decade; however, because of climate change some POPs are released back into the environment [4]. Due to their lipophilic characteristics, POPs bio-accumulate in adipose tissues putting human and wildlife health at risk [1].

The male gamete has been shown to be susceptible to damage caused by environmental toxicants such as dichlorodiphenyltrichloroethane (DDT) [5]; furthermore, studies have shown that POPs exposure impairs sperm parameters [6–8], DNA integrity [9] and chromatin condensation [5]. With respect to the sperm epigenome, DNA methylation can be altered by POPs as was showed previously by a permutation analysis in rats; and flow cytometric immunodetection and PCR pyrosequencing in men [10, 11]. Furthermore, experience-dependent information may potentially be transmitted via sperm small non-coding RNA, such as microRNA (miRNA), from the father to offspring [12, 13].

Most studies focus on the effects of individual POPs; however, humans and wildlife are exposed to complex POPs mixtures. We hypothesized that in utero exposure to an environmentally relevant Arctic POPs mixture alters the sperm epigenome, specifically miRNA expression, across multiple, unexposed generations (F1 through F4).

In addition, we investigated whether a nutritional intervention, folic acid (FA), could counteract these multigenerational epigenetic changes. Folate functions as a methyl donor in the methyl cycle, which is vital during prenatal development when epigenetic reprogramming occurs; an embryo developing under an insufficient folate status may be vulnerable to methylation-dependent epigenetic errors [14, 15]. Therefore, we hypothesized that FA supplementation moderates the POPs-induced dysregulation of sperm miRNA expression in F1 through F4 generations. Using a four-generation rat model (Fig. 1), we analysed the paternal lineage of sperm (F1–F4) derived from treated F0 dams by miRNA deep sequencing (miRNA-seq).

Methods

POPs Mixture

The POPs mixture (Table 1) represents the pollutant composition found in Ringed seal blubber of Northern Quebec which is a traditional food of Inuit people in that region [16, 17]. Mixture components were dissolved in corn oil (Aldrich-Sigma, Oakville, ON, Canada) to obtain a stock solution of 5 mg polychlorinated biphenyls (PCBs)/ml corn oil including remaining POPs, that was kept in the dark at room temperature (Table 1). The experimental dose, which is considered environmentally relevant, was made by diluting the stock solution with corn oil to a concentration of 500 µg PCBs/kg body weight as previously described in [6]; concentrations of the other POPs can be calculated from proportions listed in Table 1.

Animal Studies and Breeding

Animal care and all treatment procedures were in accordance with the guidelines of the Canadian Council on Animal Care and approved by the Université Laval animal Research Ethics Committee (certificate No. 2015010-2). Forty-five-day-old female outbred Sprague Dawley rats (Charles Rivers Laboratories, Saint Constant, QC, Canada) were housed two per cage in standard rat cages under controlled lighting (12 h light-dark cycle), humidity (46 ± 10%) and temperature (22 ± 1°C). After 10 days of acclimatization, female rats (F0 founder dams) were randomly assigned to four treatment groups (n = 6) designated ‘Control (CTRL),’ ‘Persistent Organic Pollutants (POPs),’ ‘Folic Acid supplementation (FA)’ and ‘Persistent Organic Pollutants + Folic Acid supplementation (POPs+FA)’ (Fig. 1). F0 founder dams were gavaged with the POPs mixture corresponding to 500 µg PCBs/kg body weight [6]; or corn oil (CTRL) thrice weekly and were fed the AIN-93G diet [18] containing either 2 mg/kg (1X) or 6 mg/kg (3X) of FA (Nos 110700 and 117819 Dyets, Inc., Bethlehem, PA) ad libitum. Experimental diets represent the North American FA intake in the post-fortification era (1X) and in combination with a daily 1 mg FA prenatal multivitamin (3X) [19]. Treatments were only administered to F0 founder dams for 9 weeks in total; 5 weeks before mating to untreated males at postnatal day (PND 90) and until parturition. Subsequent lineages, F1 through F4, were neither exposed to POPs nor 3X FA—instead they received 1X FA diet ad libitum.

To maximize genetic diversity, F1 male offspring, descended from different litters, were randomly selected (two per litter) to establish subsequent generations for each treatment lineage. At PND 90, F1 males (n = 12) from CTRL, POPs, FA and POPs+FA were bred with untreated females (PND 70) to obtain the F2 lineage. Likewise, F3 and F4 generation lineages were generated. At PND 150, F1–F4 males were anesthetized with 3% isoflurane and sacrificed by exsanguination via cardiac puncture followed by CO2 asphyxiation.

Sperm Isolation

Sperm were recovered from the caudal epididymides of F1–F4 Control, POPs, FA and POPs+FA male rats (n = 12) as follows: dissected caudal epididymides were placed into prewarmed Gibco Medium-199 without phenol red (Life Technologies, Burlington, ON, Canada), nicked several times using a scalpel, and incubated at 37°C while gently agitating to allow sperm to diffuse from the epididymis. After 30 min, diffused sperm were centrifuged at 2500 × g for 10 min at 4°C. Supernatant was removed, somatic cell contamination was avoided by washing the sperm pellet twice with hypotonic buffer (0.45% NaCl w/v) and centrifuged at 2500 × g for 5 min at 4°C.
Subsequently, the sperm pellet was washed twice with cold 1X phosphate-buffered saline (PBS) and centrifuged at 2500 g for 5 min at 4°C. After the second PBS wash, the pellet was resuspended in 500 ml Freezing Medium Test Yolk Buffer with gentamicin sulphate (Irvine Scientific, Edmonton, AB, Canada) and incubated for 10 min at room temperature. Last, collected sperm were stored at −80°C.

RNA Extraction
To minimize the impact of individual variation within treatment lineages, sperm were pooled from four males, with each descendant from different F0 founder dams, to provide a total of 20 × 10⁶ sperm per pool; CTRL, POPs, FA, POPs + FA (n = 3 pools composed of four individuals per pool; Fig. 1). Total RNA was extracted from pooled sperm using mirVana™ miRNA isolation.
Reverse Transcription PCR

To validate F1–F4 miRNA sequencing results, reverse transcription PCR was conducted using sperm from the same individuals that were also pooled for miRNA sequencing. Multiple miRNAs (>1000 normalized read counts) with a fold-change (CTRL/treatment) of >1.5 were selected for validation. Several other miRNAs with a fold change of ~1 or ~1 were considered as an endogenous control. We normalized using the same miRNA (miR-99a-5p) for all treatments and all generations. Extracted RNA was reverse transcribed with primers using the miScript II RT Kit (QIAGEN, Toronto, ON, Canada) according to manufacturer’s instructions. cDNAs were subjected to Real Time PCR using the miScript SYBR® Green PCR Kit, QIAGEN and the following primers (QIAGEN):

| miRNA   | Primer Assay          |
|---------|-----------------------|
| Rn_miR-34c-1 | miScript Primer Assay |
| Rn_miR-16-2  | miScript Primer Assay |
| Rn_miR-30b-1  | miScript Primer Assay |
| Rn_miR-125a-1 | miScript Primer Assay |
| Rn_miR-101a-3 | miScript Primer Assay |
| miR_99a-5p  | miScript Primer Assay |
| miR_99a-5p  | miScript Primer Assay |

miRNA Sequencing

NEBNext Multiplex Small RNA (New England Biolabs, Inc., Ipswich, MA) was used to prepare miRNA sequencing libraries according to manufacturer’s instructions. Twenty-four libraries were prepared using 30 ng miRNA purified using mirVana miRNA isolation kit (Thermo Fisher Scientific, Mississauga, Canada). 3’ SR adaptors were ligated to the 3’ end of miRNA followed by ligation of SR RT primers to the 5’ end of miRNA-3’ adaptors, which was further used for the reverse transcription step. Subsequently, 5’ SR adaptors were ligated to the 5’ end of miRNA. Following reverse transcription, an amplification for 13 cycles was performed to incorporate specific indexes for multiplexing. After purification using GenElute PCR clean-up kit (Sigma-Aldrich, St. Louis, MO), the appropriate range of cDNA fragments (120–150 bp) was extracted on a 5% gel using a Pippin Prep instrument (Sage Science, Beverly, MA). Samples were quantified using a QBit 3.0 fluorometer (Thermo Fisher Scientific, Mississauga, Canada). miRNA libraries were pooled in equimolar ratio and the quality was examined with a DNA screen tape D1000 HS on a TapeStation 2200 (Agilent Technologies, Santa Clara, CA). The final length range of libraries was verified and contained only the fraction of miRNAs. Subsequently, miRNA libraries were sequenced using two lanes of a rapid run flowcell on an HiSeq 2500 system at the Next-Generation Sequencing Platform, Genomics Center, CHU de Québec Research Center, Quebec City, Canada for single read 50 bp sequencing.

Bioinformatic Analysis

The raw sequence quality was validated using FastQC v0.11.4 [20]. Quality filtration of fastq reads and adaptor removal was carried out using Trimomatic v0.35 [21] with the following options: ILLUMINACLIP:2:30:10, TRAILING:3, LEADING:3, SLIDINGWINDOW:4:15 and MINLEN:16. Trimmed sequences were converted to fasta format using custom bash script. Blast alignment was performed using blastn v2.2.31+ against the Rattus norvegicus sequences extracted from the miRBase database release 21 with the blastn-short algorithm, a word size of 4 and a maximal E-value of 0.01 [22, 23]. Blastn results were aggregated and counts were normalized using R v3.2.0 (Team, 2013). The FactoMineR package was used to produce the principal component analysis plots. Differential expression analysis was performed using the DESeq2 v1.20.0 package [24, 25].

For subsequent analysis, a statistical significance for differential expression was set to P-value ≤ 0.05, FDR ≤ 5% and miRNAs were considered significantly differentially expressed when the difference was 0.58 on the Log2 scale (−1.5 ≥ fold change ≥ 1.5) (CTRL vs. Treatment). Gene-ontology (GO) analysis was performed using Ingenuity® Pathway Analysis (IPA®). Ingenuity Systems, Inc., Redwood City, CA) and Metascape [26] to identify gene targets that were experimentally validated by TarBase and miRecords pathways and highly predicted gene targets by TargetScan.

Results and Discussion

In support of our initial hypothesis, we first demonstrate that in utero exposure to POPs altered intergenerational sperm miRNA profiles. A total of 747 different miRNAs was detected in the sperm of rats from each of the CTRL, POPs, FA and POPs+FA lineages in F1–F4 generations (Fig. 2), of which a total of 91 miRNAs were significantly differentially expressed compared to CTRL (P-value ≤ 0.05, FDR ≤ 5%; FDR ≤ 5% and the quality was examined with a DNA screen tape D1000 HS on a TapeStation 2200 (Agilent Technologies, Santa Clara, CA). The final length range of libraries was verified and contained only the fraction of miRNAs. Subsequently, miRNA libraries were sequenced using two lanes of a rapid run flowcell on an HiSeq 2500 system at the Next-Generation Sequencing Platform, Genomics Center, CHU de Québec Research Center, Quebec City, Canada for single read 50 bp sequencing.

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Although others have reported inter- and transgenerational perturbation of non-coding RNAs in sperm following intrauterine injections of pharmacological levels of pesticides [30, 31], ours is the first to demonstrate that environmentally relevant ancestral contaminant exposures disrupt the sperm miRNA profile. In utero exposure to POPs+FA supplementation altered fewer miRNAs 1 (11, 0) in F1 sperm compared to POPs (10; 101, 0). It is tempting to speculate that maternal consumption of 3X FA diets may have partly protected her offspring’s sperm epigenome from toxicant-induced perturbation.
Interestingly, various developmental and disease conditions induced by POPs (e.g. neurodevelopmental deficits, altered reproductive functions and immunotoxicity) are related to oxidative stress-mediated cellular damage [32–34]. Studies in humans reported oxidative stress after accidental PCB poisoning or occupational POPs exposures [35, 36]. Furthermore, oxidative stress has been shown to alter small non-coding RNA (including miRNA) expression in somatic cells and sperm [37, 38]. The protective role of FA supplementation in the F1 sperm may be partly explained by its antioxidant activity if the miRNA changes are caused by oxidative stress induced by POPs exposure (i.e. F1 POPs males) [39–41]. If, however, the miRNA changes in POPs exposed sperm are due to an altered methylation capacity or dysregulated nucleotide synthesis or mutations, then the increased availability of methyl groups provided by FA supplementation may mitigate the POPs effect by supporting DNA repair through nucleotide synthesis. Additional studies of the interaction between POPs and FA are required.

Concerning the intergenerational effect observed in F1 and F2 due to POPs and POPs+FA, it remains puzzling how environmentally perturbed paternal miRNAs can persist across multiple generations [46]. To become heritable, parts of the sperm chromatin must escape reprogramming, leading to the possibility that sperm miRNA profiles are subsequently modified by

Figure 2: in utero exposure to POPs or POPs+FA alters sperm miRNA expression differentially. (A) In utero exposure to POPs and POPs+FA exposure display altered miRNA expression profiles, as revealed by miSeq analyses. Venn diagrams comparing the number and overlap of significantly differentially expressed (P-value ≤ 0.05, FDR ≤ 5%; |fold change| ≥ 1.5) miRNAs in POPs (purple), FA (orange) or POPs+FA (blue) compared to CTRL sperm in F1–F4 sperm. (B) Table including total number of significantly expressed (Sig. exp.) genes (P-value ≤ 0.05, FDR ≤ 5%) and the number of significantly differentially expressed (Sig. DE) genes (P-value ≤ 0.05, FDR ≤ 5%; |fold change| ≥ 1.5) that are up- or down-regulated indicated by * and † respectively due to POPs, FA or POPs+FA in F1–F4. (C) GO and pathway analysis based on miRNA-targeted genes, that were experimentally validated by TarBase and miRecords, plus highly predicted gene targets by TargetScan, for POPs (purple), FA (orange) and POPs+FA (blue). Top significant (P < 0.05) GOs and KEGG enriched pathways predicted by dysregulated miRNAs in F1–F3 are presented. Pathways are ranked by number of miRNA-targeted genes. Consistently, POPs targeted a higher number of genes for listed pathways in F1 and F2. FA and specifically POPs+FA targeted nearly as many genes implicated in similar pathways.
environmental factors [27]. There are clear examples of sperm DNA methylation that escape reprogramming and histones can be involved [29].

We performed GO analysis to identify gene targets of the significantly dysregulated miRNAs by 1.5-fold in F1–F4 (Fig. 2C).

Previous studies have shown that some POPs are endocrine disrupters and interfere with hormone-regulated processes including genital development, puberty onset and sperm production [47, 48]. Interestingly, here we show that in utero exposure to POPs particularly affected miRNAs implicated in mammary

Figure 3: combining FA with POPs counteracts the effect of POPs on sperm miRNA expression in F1–F3. (A) Sperm microRNA dynamics of all significant coexpressed sperm miRNAs (P-value < 0.05; FDR ≤ 5%) across treatments. POPs (purple), FA (orange), POPs+FA (blue) in F3–F4. A clear dilution effect can be observed after F2 until F4. In addition, compared to POPs, POPs+FA seems to alter similar sperm miRNAs but to a lesser extent, particularly in F1. (B) Graphs illustrating the Log2 Fold change of all sperm miRNAs specifically altered due to POPs (9 in F1, 29 in F2 and 10 in F3) compared to POPs+FA. Dashed line represents Log2 Fold change of 0.58 which equals a fold change of 1.5. All sperm miRNAs with a Log2 Fold change below 0.58 was considered as 'no change' thus control level. In F1–F3, we repeatedly observed the Log2 Fold change of POPs altered sperm miRNAs to be brought back towards control level by POPs+FA.
gland development \( (P = 1.97 \times 10^{-14}) \) and embryonic organ development \( (P = 1.62 \times 10^{-7}) \) in F1, whereas POPs + FA did not (Fig. 2C, left). In fact, only a few similar pathways were significantly affected by POPs and POPs + FA, such as cancer pathways \( (P = 1.04 \times 10^{-31}; \text{POPs + FA} P = 9.92 \times 10^{-5}) \), PI3K-Akt signalling pathway \( (P = 2.21 \times 10^{-21}; \text{POPs + FA} P = 0.004) \) and blood vessel morphogenesis \( (P = 1.41 \times 10^{-7}; \text{POPs + FA} P = 3.99 \times 10^{-5}) \) in F1. Although similar pathways were perturbed, POPs + FA appeared to affect fewer genes compared to POPs in F1. With regard to FA treatment alone, altered sperm miRNAs were specifically implicated in brain development \( (P = 8.53 \times 10^{-8}) \) and developmental growth \( (P = 3.31 \times 10^{-7}) \) in F1.

In F2, in utero exposure to POPs affected miRNAs in cancer \( (P = 6.76 \times 10^{-31}) \), sex differentiation \( (P = 1.29 \times 10^{-13}) \), brain development \( (P = 7.28 \times 10^{-23}) \) and reproductive system development \( (P = 1.02 \times 10^{-29}) \) (Fig. 2C, middle), similar to F1. Not seen in F1, however, blood vessel \( (P = 7.18 \times 10^{-27}) \), heart \( (P = 3.98 \times 10^{-26}) \) and kidney \( (P = 2.37 \times 10^{-9}) \) development were affected by in utero POPs exposure in F2. Several similarities were observed between POPs and POPs + FA, though, again fewer genes seemed to be affected by POPs + FA alone in F2.

In F3, ancestral exposure to POPs disrupted pathways involved in response to oxidative stress \( (P = 2.61 \times 10^{-10}) \) cognition \( (P = 5.65 \times 10^{-10}) \) and brain development \( (P = 1.44 \times 10^{-8}) \) (Fig. 2C, right). Also in F3, four pathways were affected by both ancestral POPs and POPs + FA, which was similar to in F2. Again, fewer genes were altered due ancestral POPs + FA compared to POPs alone.

No significantly affected pathways were observed due to all treatments in F4 \( (P / C20 < 0.05) \). Taken together, each treatment affected the sperm miRNA profile differently in each generation, implying different multigenerational signatures mediated by miRNAs.

Next, we identified several treatment-specific dysregulated miRNAs compared to CTRL in F1–F4 generations that were unique to POPs exposure and/or FA supplementation (Fig. 2A). In F1, nine miRNAs were treatment specific for POPs (purple), five miRNAs for FA and zero miRNAs for POPs + FA (blue). Also in F1, nine miRNAs were treatment specific for POPs (purple), five miRNAs for FA and zero miRNAs for POPs + FA (blue). Also in F1, nine miRNAs were treatment specific for POPs (purple), five miRNAs for FA and zero miRNAs for POPs + FA (blue). Also in F1, nine miRNAs were treatment specific for POPs (purple), five miRNAs for FA and zero miRNAs for POPs + FA (blue). Also in F1, nine miRNAs were treatment specific for POPs (purple), five miRNAs for FA and zero miRNAs for POPs + FA (blue). Also in F1, nine miRNAs were treatment specific for POPs (purple), five miRNAs for FA and zero miRNAs for POPs + FA (blue). Also in F1, nine miRNAs were treatment specific for POPs (purple), five miRNAs for FA and zero miRNAs for POPs + FA (blue). Also in F1, nine miRNAs were treatment specific for POPs (purple), five miRNAs for FA and zero miRNAs for POPs + FA (blue). Also in F1, nine miRNAs were treatment specific for POPs (purple), five miRNAs for FA and zero miRNAs for POPs + FA (blue). Also in F1, nine miRNAs were treatment specific for POPs (purple), five miRNAs for FA and zero miRNAs for POPs + FA (blue). Also in F1, nine miRNAs were treatment specific for POPs (purple), five miRNAs for FA and zero miRNAs for POPs + FA (blue). Also in F1, nine miRNAs were treatment specific for POPs (purple), five miRNAs for FA and zero miRNAs for POPs + FA (blue).
due to POPs+FA in F1–F3 generations were corrected or shifted towards CTRL levels (Fig. 3A).

As examples, in F1, besides rno-miR-6334, the majority of miRNAs are up-regulated due to POPs (purple) and restored or close to restored by FA supplementation (POPs+FA, blue; Fig. 3B left). This effect was more profound in F2 as 25 out of 29 miRNAs were restored by POPs+FA. Even in F3, we observed a mitigating effect by the FA supplementation on POPs dysregulated miRNAs, as 6 out of 10 miRNAs were brought back towards to control levels. Regardless of treatment, when compared to control, the fold-change intensity of the significantly altered sperm miRNAs lessened across generations, particularly after F2.

To further investigate whether in utero exposure to POPs and POPs+FA supplementation alters sperm miRNA expression transgenerationally, we identified overlapping and non-overlapping dysregulated miRNAs between generations per treatment lineage (Fig. 4A and B). Several studies have shown altered sperm miRNA expression profiles due to paternal diet/lifestyle [49–52]; however, few reported transgenerational inheritance of sperm miRNAs [13, 53]. Here, we found 3 intergenerational (between F1 and F2 generations) dysregulated miRNAs due to POPs exposure including rno-miR-6334, rno-miR-19b-3b and rno-miR-30b-5p (Fig. 4A). Interestingly, previous studies showed that the miR-30 family plays an important regulatory role in tissue and organ development, more specifically, and pertinent to our study, reproductive development [54]. MiR-30 is highly expressed in both mouse and human testis tissue and is associated with the Homeobox protein and Zn transport, which are critical for male fertility [55]. MiR-19 has been previously shown to be implicated in intergenerational inheritance as microinjection of either testis or sperm miR-19b of male mice fed a Western-like diet, into native one-cell embryos, lead to a Western-like diet-induced metabolic phenotype in his offspring [53]. No significantly differentially expressed miRNAs were altered beyond F2, therefore, no transgenerational epigenetic inheritance was induced by ancestral POPs exposure.

In utero exposure to POPs+FA supplementation intergenerationally (F1 vs. F2) dysregulated one miRNA (Fig. 4A). In contrast to the POPs lineage, the miR-30 and miR-19 families were not affected. Only miR-6334 was intergenerationally affected due to POPs+FA until F2. Little is known about the role of miR-6334, and no experimentally validated gene targets have been detected so far.

Using real-time PCR, three miRNAs were validated in sperm from the same individuals that had been previously pooled for miRNA sequencing (Fig. 4C and D). To be detectable using qPCR, miR34c-5p, 340-5p and 471-5p were selected based on their normalized read counts of >1000 and >1.5 fold change (CTRL/treatment). Nonetheless, we observed comparable results between miRNA sequencing data and qPCR data.

Lastly, concerning the phenotypic outcomes of this study, our team previously described in a corresponding, complementary study subtle but significant deleterious effects of prenatal exposure to POPs on male reproductive function and early embryo gene expression across at least three generations [56]. In that study, sperm quality and fertility were reduced in F2 and F3 males, respectively. Furthermore, the poorest pregnancy outcomes were observed in F3 males and F4 two-cell embryos had the highest number of significantly differentially expressed genes compared to untreated control animals [56].

In conclusion, this is a unique demonstration of the vulnerability of the paternal epigenome to the ancestral environment. We show that in utero exposure to environmentally relevant contaminants perturbs sperm miRNAs intergenerationally, but that the severity of perturbation decreases after the F2 generation. Moreover, this is the first report of a nutritionally pertinent intervention that can mitigate the effect of such contaminants.

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Supplementary data

Supplementary data are available at Environ Epigenetics online.

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