Review

The Behavioral Effect of Small RNA Differential Expression Induced Mammal Transgenerational Epigenetic Inheritance

Yuancheng Luo *

Posted Date: 19 June 2025

doi: 10.20944/preprints202506.1663.v1

Keywords: small RNA; Transgenerational; Mammal; Epigenetic

 doi:10.20944/preprints202506.1663.v1

Review

The Behavioral Effect of Small RNA Differential Expression Induced Mammal Transgenerational Epigenetic Inheritance

Yuancheng Luo [1]

Shenzhen College of International Education; e-mail@e-mail.com

* Correspondence: s22335.luo@stu.scie.com,cn; +86-158-8932-8718

Abstract: Background/Objectives: Recently, the phenomenon called Transgenerational Epigenetic Inheritance (TEI) is getting more and more focused. The effect of this non-mendelian inheritance is demonstrated to have a wider effect on people's health and also the behavioral change in certain animals. However, as this is a newly emerged area, much research is focused more on the invertebrate or simpler form of prokaryote which is due to the difficulty of doing animal research. In this review, I examine 192 researches and review and selected 99 as references which focus the discussion on. Experimental findings from mammalian studies will be compared and analyzed, along with a discussion of the mechanisms through which small RNAs contribute to TEI. The complex nature of epigenetic changing still confusing people and the variety of possibilities also enhance the difficulty of isolated and clarified single mechanism. Various **Conclusions**: Focusing on the sncRNA transgenerational epigenetic effect in mammals, this review article tries to synthesize the mechanism of small RNA in epigenetic effect and providing suggestion for the future researches.

Keywords: small RNA; Transgenerational; Mammal; Epigenetic

1. Introduction (Should Increase the Length of Introduction by Including a More Thorough Background Overview and a Detailed Analysis of Importance of TEI Effect)

In current-day research, transgenerational epigenetic inheritance (TEI) has garnered more intensive intention due to its intriguing properties. TEI refers to the phenomenon wherein the offspring experience certain phenotypic changes or behavioral shifts due to the paternal exposure to specific environmental factors that induced their gene expression alteration. The concept "TEI" begins with the investigation of "Epigenetics" which was first produced by Waddington's theory and generalized the way that external factors pose an effect on gene expression[1,2]. In contrast to the classical Darwin's theory and Weismann barrier theory, current research in TEI demonstrated that there is actually not gene content change that caused the behavioral change in the next generation, but the gene differential expression instead which opens a new direction for the animals evolution investigations[3].

With more research being completed, various kinds of organisms are found to demonstrate the TEI effect. The study of C.elegan begins with the paper about nematode's PIWI RNA differential expression causing the argonaut protein change which leads to the TEI of aversive behavior against the PA14[4]. After this paper, more papers are discussing the TEI transmission of the aversive behavior through generations which proposed more and more mechanisms and factors that include the Cer-1 transmission, nitrogen assimilation, small RNA, and bacteria non-coding RNA[5-8]. At the same time, other organisms were also found to demonstrate the TEI effect when facing environmental stress. The rice species that have TEI effect due to the heavy metal stress to the drosophila and S.pombe[9-12]. Different kinds of environmental stress are also being investigated, including

 doi:10.20944/preprints202506.1663.v1

common environmental stress such as heat, starvation, pathogenetic effect, ethanol exposure to the stress that may solely be faced by specific kinds of animals such as maternal womb pressure, and social stress including faced by mammals[13,14].

Although increment in article number about transgenerational epigenetic inheritance marks a concrete evidence for its presence and also a further investigation in its mechanism, other researchers hold the opposite idea and questioning the validity for its existence and debate around the extent of its effect on the animal's trait change. The two epigenetic reprograming events occur in the primordial germ cells and in the early embryo which makes the environmental effect transgenerational epigenetic inheritance hard to take place and post stronger effect across more than one generation[3]. Also, some researchers have proven that for certain effect previously reported as transgenerational effect is actually intergenerational interaction which further post question on how to classify the observance as transgenerational effect and whether this is a routinely events or just due to random mutation[15]. Specifically, some researchers report that the seemingly transgenerational effect of the stress on the chromatin structure and the corresponding increment in the offspring's performance or ability still lack defining evidence to prove the existence[16]. Also, most environmental stress for mammal is not strong enough to induce the transgenerational effect which leads to the proposal for the further consideration during the experiment design and analysis to ensure enough stress exposure causing substantiate effect[16]. At the same time, the research on the triclosan (TCS) effect on the newly-birth and mature flea demonstrate specific range (50-100 μg/L)of environmental stress--toxin concentration—can leads to response of the flea species transgenerically and different component of cells have different lower bound for response to generate[17]. Other than the animal, in the plant like *H. arabidopsidis,* the previously proposed transgenerational effect evidence of priming effect to the resistance to biotic stress is not rectify based on the further investigation of b-amino-butyric acid (BABA) concentration within the plant seeds which manifest the apparent deviation from the general trend[18]. The low concentration of the molecules in seeds is not high enough for transgenerational effect to occur and the similar effect is also observed when lacking the BABA molecules within the plants[18]. The examples above demonstrate the instability nature of the TEI effect in organism, warning the researchers that confirmative evidence about transgenerational effect is required for the determination of the TEI effect but not other mechanisms instead.

As all the papers indicate, there are three basic kinds of mechanisms for TEI research to be focused on, which are, DNA methylation, small RNA differential expression, and histone modification. These mechanisms make up the most prominent mechanism that is focused on which is discussed by lots of review articles and research papers. The DNA methylation and histone modification method is directly worked on the chromatin architecture to change their expression pattern which involves the interaction of the histone and DNA especially on H3K9 which is a common initiator that active in the DNA transcription[19]. In this review article, we will focus on the effect of small RNA on mammal TEI occurrence which regulates the TEI in another way by affecting the RNA-dependent RNA polymerase (RdRP) which seldom being stable in mammals as they encode it[20]. Within the scope of the small RNA mechanism, several kinds of small RNA are focused on by the researchers, including piwi interacting-RNA(piRNA), small interfering RNA(siRNA), microRNA(miRNA), small tRNA(tRNA), small nuclear RNA(snoRNA), which all of them affects the gene expression by cooperating with the DNA interference complex to block the transcription from happening to account for gene differential expression in germ-line cells or oocytes[21,22].

In this review article, the discussion will mainly focus on the mechanism of small RNA causing the TEI effect in mammals and compare the results of different mammal experiments that focus on various environmental stresses and small RNAs. The results from other experiments will be analyzed and the extent of environmental effects on animals will be focused on. This article also discusses the potential difficulty of doing mammal experiments and the possible future direction in this large area. The reasons for doing TEI-related experiments on mammals will also be presented to encourage more researchers to turn on mammal experiments in this area.

 doi:10.20944/preprints202506.1663.v1

2. Small RNA Functions and Mechanisms (Should Added More Reference to the Table Included, Should Added One or More Graph)

To begin the discussion of mammal TEI occurrence due to the small noncoding RNA effect, the focus should first shift towards the mechanism, biogenesis, and function of the common types of small RNA that produce the TEI effect to get a better understatement for the later part of this review. In this section, different kinds of small RNAs including piRNA, miRNA, siRNA, snoRNA, and tsRNA will be discussed about their different functions and the potential role in the TEI of mammals. For each small RNA here, a few examples would connect them with the future TEI effect discussion that provides a brief overview of their role in different mammals' experiments. The table below describes the basic mechanism and function of the various small RNA in the animals and the related example in TEI mechanism.

Table 2. This is a table. Tables should be placed in the main text near to the first time they are cited.

Small RNA	Small RNA mechanism	Example
piRNA	Ranging from 24-32 nucleotides in length, piRNA expresses in several types from PIWI1 to PIWI4 in mammals. Work with the argonaut protein (AGO) regulating the mammal epigenetic. Forming piRISC to recognize the mutating transposons and distinguishing between self and non-self-transposons.	Moore et al.[8] Aravin et al. [23]
siRNA	siRNA is a 21-23 nt long double-stranded RNA molecule, the siRNA complex arising when dsRNA is cleaved by Dicer, a member of the RNAase III family. The siRNA induced in RNA-induced silencing complex (RISC) interacts with Argonaut 2 component, resulting in duplex unwinding and degradation of passenger strand.	Posner et al.[24] Xu et al. [25]
miRNA	miRNA has approximated 22 nucleotides in length deriving from the longer primary miRNA transcripts. The primary miRNA will under cleavage of the RNase III Dicer, producing miRNA interacting with the Argonaut protein family. Collaborating with the Argonaut protein, miRNA will bind with the complementary DNA strands that function to repress the transcription process.	Crisóstomo et al.[26] Baldini et al. [27]
snoRNA	snoRNA is small RNA that widely presents in the nucleoli of the eukaryotic cells, ranging from 60-300 nt. snoRNA has several different types like H/ACA box	Ma et al. [28] Liu et al. [29] Sarker et al. [30]

 doi:10.20944/preprints202506.1663.v1

		snoRNA, C/D box snoRNA, small cajal RNAs. snoRNA forms the small nucleolar ribonucleoproteins (snoRNPs) by binding with structures like Cbf5p, Gar1p, and Nop58p. 2'-O-methylation and pseudouridylation will be normally involved in the functioning mechanism

2.1. Piwi Interacting-RNA (piRNA)

To begin with, the basic mechanism of piRNA should be understood for future discussions on the effect of piRNA-related TEI in mammals. The main function of piRNA is regulating the transposable element in the human genome which may lead to mutation if not regulated properly[31]. To achieve this function, the human body designs an intricate system for regulating the TE by enabling the piRNA clade to work with the argonaut protein (AGO) also known as PIWI protein for the regulation of the TE. The complex would form the piRISC complex which functions in the soma and germ-line cell to silence the target complementary RNA and inhibit the functioning of TE[32]. The place where piRNA functioning are mainly the animals' germ-line cell where the piRNA is reported to function as an important regulating factor[33]. In the animal's oocytes such as the macaque monkey, the piRNA production has a small variation of less than 10% compared to the piRNA in macaque monkeys' testicular but there is variation in piRNA length in the human testis and ovaries. Moreover, the examination of the differential expression of transcription factor A-MYB is reported insignificant in the ovary while expressed strongly in the testis of macaque monkeys and humans[34]. In mice, there are mainly three kinds of PIWI-protein, including PIWIL1, PIWIL2, and PIWIL4 all of which serve to express in different stages and places during cell development and differentiation for the controlling purpose[35,36]. The production of piRNA is also different from other small RNA which is verified as a DICE-independent process as indicated by its 24-to 30-nt length strand[37].

The loci that produced piRNA are called clusters due to the high density of piRNA sequence presence in there these sequences will be as same as another genome sequence of the mammals' RNA polymerase II, but different from drosophila as the piRNA precursor in drosophila in heterochromatic loci[21]. For piRNA in both kinds of animals, the precursor will appear in two forms the first would be the traditional 'Uni-strand' condition which the transcription will be in uni-direction, however, in the 'Dual-strands' condition, the transcription will be convergent which is identified in lepidopterans as the nearly 70% of the splice site would be in the trans-splice form in C.elegan[21,38].

Even though the piRNA performs a critical function in the animal's body the sequence is not highly conserved but demonstrates various lost and gained phase alterations during the revolution[21]. This may indicate the potential of piRNA in regulating the epigenetic inheritance of animals which may lead to the TEI as consequence. Due to the close relationship between the piRNA and TE regulation, the piRNA may lead to a large effect on the TEI effect in animals across different species. From the piwi argonaut protein regulating C.elegan to the mice that exhibit decreased social ability and social recognition due to the decreased piRNA counts[4,39]. The piRNA may also induce the TEI through modulating the functioning of the DNA methylation which was demonstrated in one study on the dnmt3 mutant drosophila that reported the piRNA effect MILI protein blockage the transposon methylated process and hence affected the overall transcription in germ-line cell[23]. The result in this study indicates the interconnection in MILI and MIWI protein associated with piRNA which both demonstrate deficiencies due to mutation as well as the MILI/MIWI2 ping pong cycle observed in the experiments may also lead to potential effect on the animal's metabolism in offspring[23]. Moreover, research has also demonstrated the role of piRNA regulation in sperm histone-to-protamine of male infertility which may also be a future direction for the research of TEI effect on mammal[40]. This could be further traced back to the PIWI effect on the MIWI binding

 doi:10.20944/preprints202506.1663.v1

protein that leads to male infertility and due to the removal of APC/C in the late spermatogenesis[41]. All these researches demonstrate the potential function of piRNA in regulating the mammal TEI effect due to environmental stress which is further verified by the instability demonstrated by the piRNA in evolution history.

2.2 Small Interfering RNA (siRNA)

The effect of siRNA in the TEI and spermatogenesis of the male is the most well-studied species of small RNA within the large category. siRNA is a 21-23 nt length double strand molecule that mainly regulated the posttranscriptional function that derived from the cleavage by Dicer protein towards the dsRNA[42]. The siRNA family could be substantially divided into two groups which are exogenous siRNA and endogenous siRNA. The main focus of TEI on animals is on the effect of RNAi induced by the environmental stress and the subsequent inducing dsRNA to accomplish the change in the transcription scheme[42]. Indeed, most of the endo-siRNA is derived from the endogenous dsRNA source this dsRNA production could happen in either the viral infection or the production by animals by itself[43]. In this whole process, a protein called dicer plays an extremely important role in identifying the dsRNAs cutting them into 21 to 23 small fragments that form so-called siRNA. These siRNAs will then bind to the guide strand and form RNA induced silencing complex (RISC) to accomplish the final interruption of the RNA transcription process and regulate the expression of gene content[44]. The siRNA will then trigger the stage called post-transcriptional gene silencing which could further be decomposed into two steps including the direct sequence-specific cleavage leads to repression of translation and content degradation and the transcriptional gene silencing[44]. During the process, the RISC complex formed by siRNA will bind with its Argonaut 2 component and will hence result in the complex unwinding the target strand or binding with the guide strand to degrade the complementary shape mRNA[42]. C. elegan, for example, has 19 kinds of functional AGO protein that could be connected with the RISC complex and siRNA, the kinds of AGO protein binding, in this case, will be based on the location of the expression and specific function required to produce in the cell[45]. The endo-siRNA in C. elegan is reported to target several AGO clusters such as the WAGO cluster which the depleted endo-siRNA targeting the ALG-3, ERGO-1, and CSR-1 class genes[45]. The function of siRNA in regulating the posttranscriptional function is reported to be important in mice's oocytes which both the endo-siRNA is reported to be regulated by the inverted pseudogene and increase regulation target as the Dicer protein removed during the production site[46]. Also, in mice's oocytes, another research team verified that the siRNA didn't require the function of RNA-dependent RNA polymerase (RdRP) to function in mammal cells[47].

The extent effect of siRNA on the occurrence of TEI should be further extended beyond the scope of general regulation of gene expression. Research has identified the direct TEI effect caused by the siRNA exerted by the maternal oocyte, the influence of siRNA in this case would further extend to the offspring for future development but not constraint to temporary differential expression[48]. The effect of siRNA on the sperm DNA expression scheme should also be focused on as the male sperms have fewer gene material contributions to the overall offspring development but a large TEI effect has been observed. The study reports that the sperm siRNA content could pose an effect on the epididymal protease inhibitor differential expression within testicular tissue which caused the increase in sperm mortality due to the presence of siRNA[25]. This influence on the sperm content could be treated as the possible effect of siRNA on the offspring's health rate.

2.3. MicroRNA (miRNA)

MiRNA is the fundamental type of lncRNA which is been studied for at least 20 years since the first discovery of miRNA in the C. elegans[49]. Having been reported as highly conservative in different kinds of animals, miRNA intrigues the interest of researchers to investigate its biogenesis and detailed mechanism in regulating gene transcription and expression which already has in-depth research of its mechanism[45,50]. The importance of miRNA is hence with no need to tell in the TEI and its function should be focused on in detail to get a more comprehensive overview of its potential mechanism and effect on mammals' TEI expression. MiRNAs are 22 nucleotide RNA that are derived from longer primary miRNA and the primary mRNA is derived from the hairpin which is processed

 doi:10.20944/preprints202506.1663.v1

through the nuclear microprocessor that bound to DGCR8 and then released the precursor hairpin. As the primary miRNA is being produced, the cleavage site is like the conventional RNA processing stage with the removal of 5′-cap and polyA tails, but this production of miRNA will mainly occur through two sites-specific cleavages which are dsRBD protein recruiting Drosha cleavage (further export to the cytoplasm by Exportin 5 protein) and the Dicer cleave occur in the cytoplasm[51]. After the complex processing stages, the miRNA with 22~23 nt length would be ready for function. For the miRNA to function, they work with the AGO2 protein to form the RISC complex and then form the mature miRNA strand and bind with the complementary RNA sequence strand to pose an interfering effect on the DNA expression or transcription[22]. In C. elegans, the investigation demonstrates that ERGO-1Ips treatment C. elegant will demonstrate decreased expression of miRNA but still enriched 26G-RNAs which indicates that miRNA enrichment present in ERGO-1 Ips may be indirect due to the interaction of ERGO-1 and ALG-1 or ALG-2 on target transcripts[45]. This result manifests the close relationship of miRNA with the AGO protein and the related protein complex, which the interaction of these pathways would proceed to the final gene differential expression ultimately. One defect that miRNA has in affecting TEI is their lack of corresponding amplification mechanism that could enable their proliferation in the offspring even under the condition that lack the previously inducing environmental signal which the piRNA and siRNA both contain[52].

Even though the lack of the self-amplification process may be a potential defect for the miRNA TEI study, still numerous researches have revealed the important role miRNA plays in the TEI effect in mammals even though the interior mechanism remains unknown. One study has proposed the importance of the miR-34/449 effect in regulating the pathway of sperm to preimplantation embryo which will pass the effect of parental social instability stress[53]. The function of miRNA is not confined to the sperm or other germ-line cells, miRNA is reported to perform a function in the motor neuron generation process in the embryo which the removal of miR-17-3p generated by cytoplasmic RNAase III Dicer would eliminate its silence effect on Olig2 in p2 progenitors. Thus, the miRNA is thought to be crucial in the refinement of the spatial and cognitive ability[54]. Moreover, the miRNA is reported to affect the alteration of animal cognitive behavior due to the enrichment environment[55]. However, all the research mentioned above does not involve the pathway of soma-germline translation and how the miRNA could pass the effect to the offspring with such a small amount previously present in sperm. This question is left unsolved and should be focused on afterward.

2.4 Small Nuclear RNA and Small tRNA (snoRNA & tsRNA)

The small nuclear RNA (snoRNA) and small tRNA(tsRNA) also have a regulated function in the sperm and perform an important role in the TEI effect. SnoRNA is widely present in the nuclei of the eukaryotic cell and has a general 60-300nt length. snoRNAs are mainly encoded by intronic regions of both protein-coding and non-protein-coding genes. SnoRNAs could be mainly classified into three groups: H/ACA box snoRNAs, C/D box snoRNAs, and small cajal RNAs (scaRNAs). The former two types of snoRNAs participate in the processing of ribosomal RNA (rRNA) by adding 2′-O-methylation and pseudo uridylation modifications to rRNA molecules which both could have potential effect on the gene expression[56]. The C/D box snoRNA has a length range from 70 to 120 nt which contains two conserved strains one C box and one D box and contains the RUGAUGA sequence in the 5′-end. Working together, these structures would intertwine to form a kink-turn which is recognized by snu-13p and then recruit the Nop1p, and Nop58p to perform the methylation modification process[27]. For the H/ACA box snoRNA, usually has 60-75 nt in length and contains the pseudouridylation pocket that functions to isomerize the uridine residue on the RNA this process could be accelerated by binding the H/ACA box snoRNA with the Cbf5p, Nop10p protein to form the complex. H/ACA box snoRNA is also similar to the C/D box snoRNA that has the H box and ACA box as conserved areas in the cells of eukaryotic organisms[56,57]. The last type of snoRNA is the scaRNAs which get its name from its location in Cajal bodies in cells that also follow the C/D-H/ACA classification but contain both structures at the same time enabling it could bind to all the proteins that could bind with the other two kinds of snoRNA[58]. The functions of different kinds of snoRNA should also be focused. Currently, the researchers have mainly identified several potential

 doi:10.20944/preprints202506.1663.v1

roles of snoRNA which are pseudouridylation, 2′-O methylation, N4-acetylcitidine, and regulation of alternate splicing[56,59]. For the N4-acetylcitidine (ac4C), it performs to increase the efficiency of translation and stability of mRNA by ensuring correct reading of codons. The GCN5-related NAT family of histone acetyltransferase NAT10 will catalyze the formation of ac4C on rRNA, tRNA, and mRNA. The ac4C will bind to the wobble position stabilizing the C3′ endo conformation of ribose, the ac4C at the wobble position will significantly promote translation efficiency and fidelity[60]. All these potential functions may contribute to the gene regulation within the cell and snoRNA plays key roles in many of these functions which would further manifest the potential of snoRNA in regulating the TEI expression in mammals and other animals that have proven to be needed for future studies by researchers in this area[60,61].

In the case of tsRNA, it is a kind of small RNA that derives from the mature tRNA which could classified into two types: tRNA-derived stress-induced RNA (tiRNA) and the tRNA-derived fragments (TRF) which both are not randomly fragmented pieces on the tRNA but function in the cells[62]. For the case of tiRNA, it is produced by the ANG cleaving at the middle of the anticodon loop of mature tRNA this cleavage would result in a 31-40nt length tiRNA that the cleavage is induced by the presence of environmental stress such as heat shock, starvation, oxidative stress that has a direct relationship with the generation of TEI[63,64]. Due to the presence of cleavage, the resulting tiRNA could be further classified into two types which are 5′ tiRNA and 3′tiRNA the 5′ one refers to the 5′ anticodon loop of tRNA towards the cleavage site, and the 3′ one vice versa[29]. For the TRF, it could be classified into 5 types which are tRF-1, tRF-2, tRF-3, tRF-5, and tRF-i. Except for the TRF-1 cleavage on the pre tRNA, the rest of the TRFs are all cleavage by one of the ANG, Dicer, or RnaseZ on the mature tRNA on different sites that would hence generate various results. The function of tsRNA is also important in gene expression regulation as the experiment reports the tRF could exhibit the mRNA-like function that can bind with the AGO proteins to form complex and bind to the 3′ untranslated area of mRNA to inhibit the translation process and hence perform the gene expression regulation function[65].

Both tsRNA and snoRNA functions in the TEI mechanisms were reported by several experiments[28,30].

3. Mammal Experiments

3.1. Mammal Experiments Problem and Difficulty

In this section, the problem and difficulty related to mammal experiments will be discussed. The reason for many experiments targeting plants and nematodes is the difficulty of mammals to demonstrate apparent behavioral change and the relatively complex mechanism behind the TEI. Due to the complex system mammals have, it is generally requiring a large degree of environmental change to cause an apparent effect on mammal behavior as studies demonstrates that animals will perform a milder behavioral change under environmental stress and some of these behavior changes are derived from their learning ability rather than the inheritance from the paternal and their passage could hence be explained as a social effect like parents teach their offspring[66]. Also, mammal TEI occurrence, requires the participation of lots of different mechanisms and pathways which adds to the complexity of the whole investigation, it is hard for researchers to focus on a single mechanism as multiple TEI pathways may be chosen by mammal bodies and the complex life cycle brings more opportunities for potential pressure effect to contact to mammals that not consider as an epigenetic pathway. Like the maternal womb pressure the mammals' embryos have a chance to contact with outer environment pressure that exerts on mammals which could hence disturb the result of the paternal side TEI investigation[13]. Furthermore, the mammal experiment has some disadvantages against the invertebrate experiments as mammals are generally harder to raise and require more specific environmental conditions to produce the individual that is ideal for the investigation this production would also require a longer cycle which would further increase the cost of experiments

To solve this problem, researchers need to consider more holistic aspects when designing the experiments, they should regulate the duration and period that mammals are exposed to environmental stress carefully to minimize the non-genetic effect on offspring especially control the pressure exposed to females when pregnancy. The researchers may need to focus on wider aspects

 doi:10.20944/preprints202506.1663.v1

when conducting the experiments and put the target investigation on more different mechanisms that possibly lead to behavioral change. The degree of environmental stress needs to be considered as well and the stress should be large enough to cause enough change in mammals' behavior cross-fostering settings may also be used to minimize the social effect on behavioral change.[67]. Moreover, the difference between the maternal and paternal effects on TEI should be focused on as the oocyte will generally contain more mitochondria and small RNA within it as TEI-induced factors, the same period exposure for both female and male mice may result in a larger effect on female mice offspring which studies demonstrate the relationship of UPRmt and BGP-15 inducer and the mitochondria RNA (mtRNA) content in female mice[68,69]. Indeed, some problems may not be able to be solved like the cost of the experiment, but it doesn't block the way of future experiments as they could produce more results in one experiment by examining wider effects to achieve more results and limiting the number of experiments to conclude inner mechanisms.

3.2. Important Mammal Experiment Comparison and Analysis

As we already discussed the mechanism of specific kinds of small RNA on TEI, in this section, the focus will shift to the experiments that investigate the TEI effect act on mammals of different species. The results of the experiments will be evaluated and compared with others in this section to provide a better overview of the TEI effect on mammals and would provide a possible future research direction for the researchers to carry on or doing refinement on the current investigation.

3.2.1. Mice Related Experiment (Should Increase the Content in Mice Related Experiment)

Most experiments related to the mammal use the mice as models as it is universal with standard gene content and traits and also easy to feed and monitor the behavior. These experiments include various kinds of environmental stress and demonstrate the different kinds of mice's behavioral or physiological changes when facing these factors. In each experiment, the mice's behavior is being assessed and the number of generations that possess the trait is being examined as one of the factors that influence the extent of environmental effect as well as the difference of the trait demonstrated from the normal behavior. Based on the different environmental factors, it could be classified into positive environmental stimulation, also known as environment enrichment (EE), or the negative environmental stress exerted on the mice. Also, for these experiments, the function of small RNA in each case is being investigated and the comparison of the result would give a more holistic overview of this area of research.

For the positive environmental stimulation, there are relatively fewer cases compared to the negative environmental stress as the positive one is generally concerned as cannot exert much effect on the gene content of the mice offspring. One of the positive environmental stimulation investigations is the effect of EE on improving mice's intergenerational cognitive behavior change[55]. In this research, the researchers demonstrate that the mice will perform enhanced long-term potentiation (LTP) increase in the hippocampus area due to the upregulation of miR212/132 and other targeted miRNA in the sperm. The mice who are the offspring of the EE have higher cognitive scores compared to the control group[55]. The ability of TEI and small RNAs to increase cognitive ability is further manifested in another experiment in which the researchers investigate the effect of environment enrichment on alleviating the mutant effect on LTP of the mice and their offspring. Their result indicates that the EE could help to decrease the mutant effect on LTP for several generations and this effect is decreased as the generation progresses the contextual memory could also be increased by the EE and have the same trend with the LTP across the generations[70]. Similar to the EE, some researcher's investigation of the social enrichment after the postnatal phases could also have transgenerational effects such as increasing nursing contact on mice that last 3 generations from F0 to F2[71]. Especially for the female offspring, the researchers observe the presence of decreased stress levels and increased social interaction with other mice within the communal and the researchers attribute these changes to increased oxytocin levels. As this behavioral change is still present in the offspring it may be due to the differential expression of gene induced by the small RNA in the genome[71]. Despite the common cognitive improvement ability, EE is also reported to prevent the transgenerational effect of parental trauma in the mice's offspring which the alteration of gene

 doi:10.20944/preprints202506.1663.v1

methylation and small RNA may lead to this change in mice epigenetic expression[72]. Like the EE, the physical exercise along on mice is reported to have increase brain cognition function which demonstrated through the improving score of Novel Object Recognition Test (NOR) and this phenomenon will pass transgenerationally across generation from F0 to F1[73]. This paternal side transmission is found to associate with the increasing expression of cell proliferation gene sequence and also the miR212-132 sequence in both the offspring and parent who have more exercise compare to the sedentary group[73]. Also, the environmental enrichment like attributed more exercising opportunities for the mice enable them to present increasing memory recall and also spatial and non-spatial information processing ability[74]. For the group of F2 generation of mice from the parents who not exposing to exercise restraint, the higher performance was observed during the NOR, CFC and OL tests which demonstrate more ability to explore novel object and accepting the new knowledge[74,75]. In the smallRNAseq of the F2 generation hippocampus, an underregulation of the 35 sDE microRNAs and 15 microRNAs and the upregulation of 20 microRNA is recorded in more active mice F2 generation compare to the sedentary one[74].

Overall, the positive environment stimulation would present an increase in cognitive behavior and hippocampus ability in the brain of mice and this effect could last for more than one generation which is induced by either small RNA or the DNA methylation method. However, these experiments' results still have some defects in that they didn't include the detailed mechanism of how the inner cell process is altered by the epigenetic factors and this lack of detailed mechanism could only provide us with a limited overview of the EE effects on mammals which would hence require more research to examine the various factors that could lead to the alteration.

Although the effect of positive environmental stimulation is promising as the potential to be used for human cognitive behavior enhancement, the extent of this kind of environmental influence is limited compared to the more general environmental stress. As most of the positive environment stimulation only lasts for intergenerational or only 2 to 3 generations, the effect of environmental stress such as the pathogen threat could even pass the influence to at most 5 generations in the C.elegan[4]. For the case of mice, the general TEI effect induced by the environmental stress could be extended from 4 to 5 generations which have longer duration compared to the positive environmental stimulation[76]. Lots of studies demonstrate the effect of negative environment stress on the mice that each stress would induce the response from experiment subject uniquely and differentiate from each other's which each response would involve one or more kinds of small RNA participants. The tsRNA is reported as very important in the TEI occurrence of the mice as it would directly function in the mice sperms and regulate the gene expression there[29]. It is reported to function in the TEI effect due to environmental stress such as parental high-fat diet (HFD) and its upregulation leads to apparent change in gene content as accompanied by the upregulation of the miRNA[26]. In the experiment, the alteration of expression in the G-protein signaling pathway is observed in the F1 and F2 generations and this alteration is coupled by the upregulation in tsRNA which results in the effecting in the testicular metabolism function. Except for the tsRNA, other piRNA and miRNA are also differentially expressed in the F1 and F2 generations but the extent of alteration is not significant compared to the tsRNA. The researchers in this experiment measure the extent of the HFD effect by measuring the GO term differential expression and the intergenerational expression change is observed with a limited passing length[26]. Also, another study manifests that the maternal overnutrition program could cause the tsRNA expression alteration in the male sperm of mice which would lead to obese phenotype in the later generation[30]. The research team observes the differential expression of tsRNA targets like CHRNA2 and GRIN3A both of which imply addiction pathology which hence explains the behavior change demonstrated by the mice. The researcher further verified this effect by injecting the pre-made tsRNA into the embryonic F1 mice obesogenic and addictive behaviors were observed in the F1 mice that were similar to the F0 generation that experienced the HFD program hence verifying the effect[30]. In both cases, the research teams observed the alteration in tsRNA contents under the HFD condition which the mice generations they observed led to different behavior changes the first experiment demonstrates the increased stress behavior and the second demonstrates the increasing additive behavior. Crisóstomo et al. in a later examination state that the alteration of tsRNA and other sncRNA due to the HFD would result in the defect of sperm

 doi:10.20944/preprints202506.1663.v1

content and hence would cause a transgenerational effect on the later generation[26]. Except for the tsRNA alteration under HFD environmental stress, other kinds of sncRNA such as miRNA are reported to function to help the gene differential expression under the HFD environmental stress[77-79]. In these experiments, the differential expression of microRNAs such as miR-149-5p, miR-335-3p, miR10b-5p, and miR-122 would induce the change in lipid metabolism scheme and also the glycolipid metabolism in the offspring within several generations. Thus, the mice's metabolism and body function will be affected by various kinds of pathways and this would eventually result in different results even for similar pathways.

Except for the HFD stress and the resulting behavior change, other environmental stress is also critical for focusing. In one study, the researchers found that environmental psychological stress would lead to a metabolic change in the offspring, and this change is due to the differential DNA methylation in the sperm this different level of methylation is reported to be induced by the function of miRNA[80]. Also, the paternal environmental exposure can cause the alteration in spermatozoa sncRNA alteration and causing the next generation with multiple disease and other health condition[81]. In one study, the alteration of expression of sperm microRNA is reported to effect the depression-like syndrome susceptibility in the mice which the distinct expression profile in F0 generation depression mice will be passed to the F1 generation using the sperm as vector[82]. During the test, the F1 generation of the depressive offspring express similar symptom as their parents like longer floating time in the water and lower sucrose ingestion compare to the control group of F1 mice. For the expressive of the depression symptom, large increase in upregulation of the sperm miRNA is observed while the piRNA experience significant decrease in expression intensity compare to the control group for both F0 generation and F1 generation[82]. Another research also demonstrates the TEI effect could be the result of the function of different kinds of mechanisms in which the DNA methylation, histone modification, and piRNA differential expression both work together to lead to the inherited effect of the DDT-induced TEI effect[83]. Using the RNA-seq analysis, the location of ncRNA differential expression is demonstrated to be distinct with each generations from F1 to F3, but specifically, the F3 generation demonstrate more similarity with the F1 generation with 29 locations of repeating demonstrate the transgenerational effect presented by mice[83]. The real world pM2.5, also, is founded to affect the mice sncRNA especially the microRNA and piRNA expression which cause the hypogonadism in the offspring transgenerationally[84]. Specifically, the miR6240 and piR016061 is responsible for this change, which accompanied with other 19 piRNA and 3 tsRNA that differentially expressed within the male sperm and 13 of them upregulated while other experience down-regulation[84]. When mice exposing to other toxicity like environmental stress, like the heavy metal cadmium, alteration in the expression scheme of tsRNA expression which 9 is upregulated and 5 is down regulated is observed within mice and the following change will pass to the next generation[85]. The change in tsRNA expression reflect further on the development of mice's mitochondria and the lysosome within the testes and liver. The reported decrease activity of content of mitochondria in 2-cell stage and morula stage is accompanies with the increasing expression of mitochondria and lysosome in 6-days old offspring mice's testes but decreasing expression significantly in the adult stage[85]. For the pathway involves in coordinating with the cadmium exposure stress, the neuro-interactive ligand receptor pathway is reported with most significant related with this trait change within the mice testes[85].

When comparing these results of research, the positive environment stimulation is demonstrated to have less implication on TEI of the mammal behavior alteration and most studies on the negative environmental stress could extend the effect to several generations. For each case of mice experiment, there are multiple small RNA participants for the overall TEI effect on the offspring or several generations later. Some are upregulated and others are downregulated and the gene expression they regulate works together to change the behavior and metabolic effect of the offspring. These experiments reveal the complexity of the TEI effect and also remind future researchers to increase the variety of research targets. The effect of TEI on different parts of the organism should also be focused as it demonstrates the possibility.

 doi:10.20944/preprints202506.1663.v1

3.2.2. Human Related Experiment & Research (Also Included Data Analysis from Human Related Experiment and Research)

For the human experiments, there are relatively fewer cases compared to the mice due to the ethical issue of conducting experiments on the human bodies. Along with the ethical issue, the difficulty in operating the experiments like precisely controlling the F1 or F2 generation exposure time added more difficulty to the experiments[86]. Also, the lack of providing sufficient mechanistic insight into human evidence blocks the way for further investigation. Along with the evidence from humans is mostly from non-invasive methods like blood and urine, they are inadequate for the studying of complex epigenetic dynamics that occur during development[87]. The relative deficiency in understanding the human epigenetics transmission effect blocks future experiments and reviews from finding basements to support theories. Even though few experiments are conducted, these experiments have made some progress as they demonstrate the TEI effect could also affect human and their offspring. The most famous human experiments would be the observance of the TEI effect on the offspring of people who experienced the Netherlands famine demonstrates a higher chance of having chronic diseases and metabolic diseases in adulthood compared to the siblings who have not experienced the famine[88]. Several other studies have also demonstrated the effect of paternal poor on the food supply in children's childhood can lead to an increase in the probability of cardiovascular disease[89]. Another experiment on humans focused on the TEI effects caused by the polycystic ovary syndrome(PCOS) of the maternal side would pass the reproductive and metabolic phenotypes to the male offspring which has been manifested both in humans and the mice[10]. Furthermore, other experiments demonstrate the potential effect of non-DNA transgenerational epigenetic inheritance on the passage of mental disorders to human offspring and these effects will eventually express during the pubescent time with the phenotype of increasing possibility of mental disorder[90]. Also, some related experiments demonstrate the potential of sperm transmission in the human as the rapid response to diet which provides a potential target for the overnutrition environmental stress investigation, with the reported potential effect on the sperm tsRNA upregulation due to the ingestion of high sugar diet[91]. Furthermore, when exposing to endocrine disrupting chemicals (EDC) 2,3,7,8-tetraclorodibenzo-p-dioxin will causing the transgenerational transmission effect in human granulosa cells which caused by the miRNA[92]. Using the miRNA4.0 arrays, 109 sncRNA are founded to be differentially expressed within the cells when the cells exposing the EDC at day 9 and day 14 for 2 and 72 hours of chronic exposure[92]. Specifically, the predicted differentially expressed miRNA like miR-24-2-5p, miR 30b-5p, miR 27a-3p, and miR 8063, are involved in the pathway of cancer regulation which indicated their potential role in cancer producing and pointing to the possibilities of transgenerational cancer transmission and promotion effect within human[92]. Other outer environmental stress such as smoking will also exert potential disturbance to the miRNA expression in the human embryo. For example, one research team finished the microRNA analysis has found that 28 miRNA had been altered due to the effect of smoking and may lead to different schemes of cell death and apoptosis[93].

Expanding from the nutritional effect on human epigenetic expression, the mental effect is also critical for some certain traits' alteration. Exercising in humans is demonstrated to have the effect of altering mental health conditions and curing spectrums of mental diseases like ADHD and autism[86]. Not only solely focusing on the human experiments, some researchers seek to combine the evidence from the mice and the human males. With the usage of the Adverse Childhood Experience (ACE) questionnaire, researchers using the score from ACE with the sperm miRNA, and siRNA expression result to determine that the miR-449/34 family will perform an inverse relationship with the ACE score revealing the transgenerational effect[94]. From the mice, they investigated the early chronic social instability (CSI) stress in the male mice and found similar results with the experiments from humans hence further verifying this condition[94].

The human area within the TEI research field is still requiring much effort of investigation in the future. Lots of areas are still left resolving for the human transgenerational effect which requires more data collected from either experiments or the clinical observation that involves the genetic testing or RNA expression examination.

 doi:10.20944/preprints202506.1663.v1

3.3. Different Kinds of Environmental Stress

In this part, there will be a brief discussion about the general environmental stress included in different research and the result of this environmental stress will be discussed. For the different environmental stress, each effect and behavioral change induced will be discussed and compared.

One of the common environmental stress that are being investigated is starvation or malnutrition. The parental mice will usually be fasting for several periods cyclically to mimic the famine condition. The fasting and TEI reveal the increased expression of the autophagy gene in the human liver and muscle and also the upregulation of certain small RNAs, including mtRNA and siRNA[95]. Opposite to starvation is the high-fat diet (HFD) or overnutrition environmental stress. In this condition, the subjects will be provided with a diet that has a lower percentage of protein concentration and a higher percentage of fat concentration which will, and the effect of this type of diet will be measured. Several types of research demonstrate that under HFD, mice will express subfertility for both female and male offspring of two generations of mice and will also express higher levels of glucose level within the blood accompanied by insulin resistance symptom[96,97]. The effects of HFD could be passed through generations through both DNA methylation and sperm defects in mice, both of the mechanisms leading to the presence of an increase in susceptibility to diabetes and a decrease in the health condition of offspring for the first generation[98,99]. Except for the common starvation and overnutrition investigation, other food-related experiments such as exposure to ethanol provide interesting results that the exposure to ethanol leads to increased depressive behavior, and this alteration extends to the F3 generations in 20-day-old mice which may be induced by the altered cortical. Expression of Id2 in F1 generation[14].

Another stress that is commonly investigated in mammals is the psychiatric stress of the parents. The parental mice will be exposed to long-term constraint pressure conditions that will induce depression or anxiety-like behavior in them., then the effect of this stress will be investigated in their offspring to see whether there is behavior change and related genetic expression and content. In one study, the researchers found that when parental mice were exposed to a long-term restraint stress model that induced depression and anxiety could pass metabolic and behavioral disorders like lower body weight and higher blood glucose levels after being fed to the next generations of mice. As this alteration occurs, they find differential DNA methylation regions of intergenerational at ~11.36% and also the differential expression of tsRNA, miRNA, rRNA derived small RNA (rsRNA)[80]. Other experiments also demonstrate the effect of psychiatric stress on the offspring that postnatal trauma could be passed by alteration expression of lncRNA and the parental stress may contribute to the occurrence of offspring's hypothalamic–pituitary–adrenal (HPA) axis dysregulation which using the alteration of sperm miRNA expression scheme as a conduction pathway[100,101]. The offspring is demonstrated to have a more increased level of corticosterone after experiencing 15 minutes of restraint stress compared to the control group offspring[100].

Except for the stress mentioned above, other stresses such as heat, pathogenic infection, and olfactory imprinting fear are all focused by the researchers and find different levels of effect on the offspring and different mechanisms involved pass the effect to the next generations[39,102,103]. In the case of pathogenic infection, the infected male mice are demonstrated to have behavioral change with less sex vigor and with lower sperm production, the F1 offspring also demonstrate spending less time in the center in open field tests which indicates anxiety-like behavioral alteration. In the olfactory-imprinting fear experience experiment, the F0 generation conditioned fear experience to a specific odor, and the subsequent F1 and F2 generation is demonstrated to have increased sensitivity to the odor that conditioned with fear in the F0 generation.

For all these environmental stresses, more research should be completed to give a more holistic view of the relationship between animal behavioral change and environmental stress. The experiments may also shift some focus to the more positive environment and the potential benefits it may bring to the mice and their offspring.

4. Discussion (Should Increase the Content in Discussion, Should Include Some Basic Data Analysis)

 doi:10.20944/preprints202506.1663.v1

Even though much research has been done on the mammal TEI effect due to environmental stress, current research inevitably falls into some way of bias and inclination towards the simpler form of the animals and also solely focusing on the result of TEI but ignoring the mechanism behind that cause it to happen as transferring from the soma to germline. The pathway should be more focused by the researchers especially for the soma to germ-line pathway which reveals the inner mechanism of how environmental stress affects the TEI of mammals and this feature is lacking in lots of studies. For instance, the Benito et al. study about the effect of environment enrichment on the cognitive ability of offspring lacks a detailed investigation of how the siRNA and piRNA affect the sperm cell and how they are been transported from the hippocampus to the sperm[55]. This may be due to the difficulty in conducting the mammal experiments which the long feeding and maturing period may lead to the researcher having less chance to operate on the offspring. Also, the lack of human research may also due to the difficulties for human to demonstrate transgenerational radiation across the generations because of the specific oocyte resting mechanism, the haploid insufficient amount of small RNA and gene to transmit and the specific spermatogenesis causing various in sensitivity extent which destabilize the transgenerational radiation[104]. As a result, the different aspects of mechanisms may not be sufficiently investigated. Even under these conditions, several researches have been done about the soma-to-germline transportation process. This phenomenon is first noticed in Drosophila which the neuroactive compound pentylenetetrazol leads to increased transcriptomic content in the CNS of F0 generation and this increment of transcriptome is also observed in F0 & F1 testis[105]. Another experiment concludes that environmental stress. Also, the mammal experiments tend to be more complex compared to the invertebrate experiments as the behavioral change of animals seems to have a less direct relationship with the environmental stress that affects their parents. For example, an experiment that targets the pathogenic effect on mice demonstrates the offspring of affected mice only show increased anxiety and nervous-like behavior instead of behavioral change like nematode about the direct aversion towards the source of the pathogen[4,39]. This phenomenon may relate to the complex inheritance mechanism possessed by mammals in which the affected genes may not directly contribute to the TEI effect that could increase the survival rate of offspring which demonstrated in the nematode that the F1 to F4 generations all demonstrate increased resistance and survival rate when placing with the deadly PA14[4].

As facing some current deficiencies in the area of research, several solutions could also be proposed to solve this instead of being evasive to the problem. The new RNA sequencing method could provide further investigation into the expression level of different RNA in sperm[106]. The enhanced sequencing technique would also enable the more rapid experiment processes which lower the time cost of conducting mammal experiments and the accuracy of results which has more statistical significance and directivity. Accompanied by the advances in sequencing technology, the shift in focus in this area is also important as the view is not confined to the three major mechanisms but extends to another possible pathway that could explain the same consequence. For example, the experiments on nematodes, test the mechanism of TEI effect towards the PA14 aversion range from the general piwi RNA TEI to the Cer1 role in the horizontal transfer and heterochromatin formation due to RNAi[8,107]. The current increased attention on this area also aids the generation of several review articles and meta-analyses that could provide an overview in this area of study pointing researchers to the future directions in this area and providing useful data for references at the same time[13,108-110]. Also, researchers should try to do more mammal-related experiments that have similar structures and gene sequences to humans and thus could provide more insightful implications to the human medical research area. The animals' experiments have extended to pigs, dogs, fishes, and other kinds of general rodents which would provide other researchers with a more comprehensive overview of the different TEI expressions within different functioning systems[111,112].

Thus, even though still requires much investigation, this research area is becoming more mature with a complex and intricate knowledge frame as well as a wide range of experiments on different animals from invertebrates to vertebrates. As a result, the research in this area is destined to become faster than before with more key data generated through multiple investigations and interactions of

 doi:10.20944/preprints202506.1663.v1

different datasets. The booming time of this research area will come after overcoming several key problems including how to ensure apparent behavioral change, the pathway of inter-body information exchange, and how different potential TEI systems interact with each other to produce the final result that leads to the behavioral change. These problems will be the potential future research direction in this area, and we believe they will be solved soon.

5. Conclusions

To conclude, for the research on the TEI effect induced by the small RNA on mammals and other vertebrates, the researchers are demonstrating the effect of TEI more and more thoroughly. But even for this good situation, the researchers should face the problem such as ignoring the deep mechanisms that lead to the soma-germline communication pathway and the inclination on the animal-specific TEI mechanisms instead of considering all the possible mechanisms that may contribute to the overall behavioral change. Despite the possible defect of the area of study, the meaning and the possible usage of the knowledge of this area in the future is very promising. The deep mechanism may serve as a potential target for the medical treatment of inheritance disease and could also be a factor considered by paleontologists when investigating the evolution and trait change across generations. Especially for animal experiments the inner pathway and several unsolved questions still present should be focused on. Due to the complexity of mammals' physiological mechanisms, researchers should not treat mammals as other kinds of experiment subjects that longer or larger scale of environmental stress may be implied to achieve enough effect on the offspring and both maternal and paternal sides should strictly regulate the exposure to environmental stress.

In the future, the researchers should concentrate on combining the effects of different mechanisms which give a more holistic view of the inner cause of the behavioral change of the offspring due to the environmental stress. Researchers should also try to discover new mechanisms that could serve to explain the TEI rather than basing all the studies on the three preexisting mechanisms. However, the mechanisms present today also need to be studied more closely to determine the exact route taken by the inner signal transmission pathway. Overall, this review article discusses the common pathway that small RNA TEI may involve accompanied by a brief explanation of the potential mechanism that leads to the TEI. Also, different TEI evidence on mammals is discussed which compares the experiment results.

References

1. Waddington, C.H. The epigenotype. 1942. *Int J Epidemiol* **2012**, *41*, 10-13, doi:10.1093/ije/dyr184.
2. Newman, S.A.; Forgacs, G.; Muller, G.B. Before programs: The physical origination of multicellular forms. *The International Journal of Developmental Biology* **2006**, *50*, 289-299, doi:10.1387/ijdb.052049sn.
3. Khatib, H.; Townsend, J.; Konkel, M.A.; Conidi, G.; Hasselkus, J.A. Calling the question: what is mammalian transgenerational epigenetic inheritance? *Epigenetics* **2024**, *19*, doi:10.1080/15592294.2024.2333586.
4. Moore, R.S.; Kaletsky, R.; Murphy, C.T. Piwi/PRG-1 Argonaute and TGF-β Mediate Transgenerational Learned Pathogenic Avoidance. *Cell* **2019**, *177*, 1827-1841.e1812, doi:10.1016/j.cell.2019.05.024.
5. Copenhaver, G.P.; Sengupta, T.; St. Ange, J.; Kaletsky, R.; Moore, R.S.; Seto, R.J.; Marogi, J.; Myhrvold, C.; Gitai, Z.; Murphy, C.T. A natural bacterial pathogen of C. elegans uses a small RNA to induce transgenerational inheritance of learned avoidance. *PLOS Genetics* **2024**, *20*, doi:10.1371/journal.pgen.1011178.
6. Kaletsky, R.; Moore, R.S.; Vrla, G.D.; Parsons, L.R.; Gitai, Z.; Murphy, C.T. C. elegans interprets bacterial non-coding RNAs to learn pathogenic avoidance. *Nature* **2020**, *586*, 445-451, doi:10.1038/s41586-020-2699-5.
7. Marogi, J.G.; Murphy, C.T.; Myhrvold, C.; Gitai, Z. Pseudomonas aeruginosa modulates both Caenorhabditis elegans attraction and pathogenesis by regulating nitrogen assimilation. *Nature Communications* **2024**, *15*, doi:10.1038/s41467-024-52227-3.
8. Moore, R.S.; Kaletsky, R.; Lesnik, C.; Cota, V.; Blackman, E.; Parsons, L.R.; Gitai, Z.; Murphy, C.T. The role of the Cer1 transposon in horizontal transfer of transgenerational memory. *Cell* **2021**, *184*, 4697-4712.e4618, doi:10.1016/j.cell.2021.07.022.

 doi:10.20944/preprints202506.1663.v1

9. Burns, J.G.; Mery, F. Transgenerational memory effect of ageing in Drosophila. *Journal of Evolutionary Biology* **2010**, *23*, 678-686, doi:10.1111/j.1420-9101.2010.01932.x.
10. Risal, S.; Li, C.; Luo, Q.; Fornes, R.; Lu, H.; Eriksson, G.; Manti, M.; Ohlsson, C.; Lindgren, E.; Crisosto, N.; et al. Transgenerational transmission of reproductive and metabolic dysfunction in the male progeny of polycystic ovary syndrome. *Cell Reports Medicine* **2023**, *4*, doi:10.1016/j.xcrm.2023.101035.
11. Yu, R.; Wang, X.; Moazed, D. Epigenetic inheritance mediated by coupling of RNAi and histone H3K9 methylation. *Nature* **2018**, *558*, 615-619, doi:10.1038/s41586-018-0239-3.
12. Cong, W.; Miao, Y.; Xu, L.; Zhang, Y.; Yuan, C.; Wang, J.; Zhuang, T.; Lin, X.; Jiang, L.; Wang, N.; et al. Transgenerational memory of gene expression changes induced by heavy metal stress in rice (Oryza sativa L.). *BMC Plant Biology* **2019**, *19*, doi:10.1186/s12870-019-1887-7.
13. Zhang, Q.; Tian, Y. Molecular insights into the transgenerational inheritance of stress memory. *Journal of Genetics and Genomics* **2022**, *49*, 89-95, doi:10.1016/j.jgg.2021.11.015.
14. Bottom, R.T.; Kozanian, O.O.; Rohac, D.J.; Erickson, M.A.; Huffman, K.J. Transgenerational Effects of Prenatal Ethanol Exposure in Prepubescent Mice. *Frontiers in Cell and Developmental Biology* **2022**, *10*, doi:10.3389/fcell.2022.812429.
15. Heard, E.; Martienssen, R.A. Transgenerational epigenetic inheritance: myths and mechanisms. *Cell* **2014**, *157*, 95-109, doi:10.1016/j.cell.2014.02.045.
16. Pecinka, A.; Mittelsten Scheid, O. Stress-induced chromatin changes: a critical view on their heritability. *Plant Cell Physiol* **2012**, *53*, 801-808, doi:10.1093/pcp/pcs044.
17. Lee, J.S.; Oh, Y.; Lee, J.S.; Kim, H.S. Acute toxicity, oxidative stress, and apoptosis due to short-term triclosan exposure and multi- and transgenerational effects on in vivo endpoints, antioxidant defense, and DNA damage response in the freshwater water flea Daphnia magna. *Sci Total Environ* **2023**, *864*, 160925, doi:10.1016/j.scitotenv.2022.160925.
18. Slaughter, A.; Daniel, X.; Flors, V.; Luna, E.; Hohn, B.; Mauch-Mani, B. Descendants of primed Arabidopsis plants exhibit resistance to biotic stress. *Plant Physiol* **2012**, *158*, 835-843, doi:10.1104/pp.111.191593.
19. Kim, M.; Costello, J. DNA methylation: an epigenetic mark of cellular memory. *Exp Mol Med* **2017**, *49*, e322, doi:10.1038/emm.2017.10.
20. Boskovic, A.; Rando, O.J. Transgenerational Epigenetic Inheritance. *Annu Rev Genet* **2018**, *52*, 21-41, doi:10.1146/annurev-genet-120417-031404.
21. Ozata, D.M.; Gainetdinov, I.; Zoch, A.; O'Carroll, D.; Zamore, P.D. PIWI-interacting RNAs: small RNAs with big functions. *Nat Rev Genet* **2019**, *20*, 89-108, doi:10.1038/s41576-018-0073-3.
22. Shang, R.; Lee, S.; Senavirathne, G.; Lai, E.C. microRNAs in action: biogenesis, function and regulation. *Nat Rev Genet* **2023**, *24*, 816-833, doi:10.1038/s41576-023-00611-y.
23. Aravin, A.A.; Sachidanandam, R.; Bourc'his, D.; Schaefer, C.; Pezic, D.; Toth, K.F.; Bestor, T.; Hannon, G.J. A piRNA Pathway Primed by Individual Transposons Is Linked to De Novo DNA Methylation in Mice. *Molecular Cell* **2008**, *31*, 785-799, doi:10.1016/j.molcel.2008.09.003.
24. Posner, R.; Toker, I.A.; Antonova, O.; Star, E.; Anava, S.; Azmon, E.; Hendricks, M.; Bracha, S.; Gingold, H.; Rechavi, O. Neuronal Small RNAs Control Behavior Transgenerationally. *Cell* **2019**, *177*, 1814-1826.e1815, doi:10.1016/j.cell.2019.04.029.
25. Xu, J.; He, M.; Wang, W.; Hou, J.; Chen, X.; Ding, X.; Zhang, J. siRNA-mediated Eppin testicular silencing causes changes in sperm motility and calcium currents in mice. *Reprod Biol* **2021**, *21*, 100485, doi:10.1016/j.repbio.2021.100485.
26. Crisóstomo, L.; Bourgery, M.; Rato, L.; Raposo, J.F.; Batterham, R.L.; Kotaja, N.; Alves, M.G. Testicular "Inherited Metabolic Memory" of Ancestral High-Fat Diet Is Associated with Sperm sncRNA Content. *Biomedicines* **2022**, *10*, doi:10.3390/biomedicines10040909.
27. Baldini, L.; Charpentier, B.; Labialle, S. Emerging Data on the Diversity of Molecular Mechanisms Involving C/D snoRNAs. *Non-Coding RNA* **2021**, *7*, doi:10.3390/ncrna7020030.
28. Ma, Z.; Tang, N.; Zhang, R.; Deng, H.; Chen, K.; Liu, Y.; Ding, Z. Ribonuclease Inhibitor 1 (RNH1) Regulates Sperm tsRNA Generation for Paternal Inheritance through Interacting with Angiogenin in the Caput Epididymis. *Antioxidants* **2024**, *13*, doi:10.3390/antiox13081020.

 doi:10.20944/preprints202506.1663.v1

29. Liu, B.; Cao, J.; Wang, X.; Guo, C.; Liu, Y.; Wang, T. Deciphering the tRNA-derived small RNAs: origin, development, and future. *Cell Death Dis* **2021**, *13*, 24, doi:10.1038/s41419-021-04472-3.
30. Sarker, G.; Sun, W.; Rosenkranz, D.; Pelczar, P.; Opitz, L.; Efthymiou, V.; Wolfrum, C.; Peleg-Raibstein, D. Maternal overnutrition programs hedonic and metabolic phenotypes across generations through sperm tsRNAs. *Proceedings of the National Academy of Sciences* **2019**, *116*, 10547-10556, doi:10.1073/pnas.1820810116.
31. Grundy, E.E.; Diab, N.; Chiappinelli, K.B. Transposable element regulation and expression in cancer. *FEBS J* **2022**, *289*, 1160-1179, doi:10.1111/febs.15722.
32. Wang, X.; Ramat, A.; Simonelig, M.; Liu, M.F. Emerging roles and functional mechanisms of PIWI-interacting RNAs. *Nat Rev Mol Cell Biol* **2023**, *24*, 123-141, doi:10.1038/s41580-022-00528-0.
33. Siomi, M.C.; Sato, K.; Pezic, D.; Aravin, A.A. PIWI-interacting small RNAs: the vanguard of genome defence. *Nat Rev Mol Cell Biol* **2011**, *12*, 246-258, doi:10.1038/nrm3089.
34. Roovers, Elke F.; Rosenkranz, D.; Mahdipour, M.; Han, C.-T.; He, N.; Chuva de Sousa Lopes, Susana M.; van der Westerlaken, Lucette A.J.; Zischler, H.; Butter, F.; Roelen, Bernard A.J.; et al. Piwi Proteins and piRNAs in Mammalian Oocytes and Early Embryos. *Cell Reports* **2015**, *10*, 2069-2082, doi:10.1016/j.celrep.2015.02.062.
35. Klattenhoff, C.; Theurkauf, W. Biogenesis and germline functions of piRNAs. *Development* **2008**, *135*, 3-9, doi:10.1242/dev.006486.
36. Du, L.; Chen, W.; Zhang, D.; Cui, Y.; He, Z. The functions and mechanisms of piRNAs in mediating mammalian spermatogenesis and their applications in reproductive medicine. *Cellular and Molecular Life Sciences* **2024**, *81*, doi:10.1007/s00018-024-05399-6.
37. Bernstein, E.; Caudy, A.A.; Hammond, S.M.; Hannon, G.J. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* **2001**, *409*, 363-366, doi:10.1038/35053110.
38. Gu, W.; Lee, H.-C.; Chaves, D.; Youngman, Elaine M.; Pazour, Gregory J.; Conte, D.; Mello, Craig C. CapSeq and CIP-TAP Identify Pol II Start Sites and Reveal Capped Small RNAs as C. elegans piRNA Precursors. *Cell* **2012**, *151*, 1488-1500, doi:10.1016/j.cell.2012.11.023.
39. Tyebji, S.; Hannan, A.J.; Tonkin, C.J. Pathogenic Infection in Male Mice Changes Sperm Small RNA Profiles and Transgenerationally Alters Offspring Behavior. *Cell Reports* **2020**, *31*, doi:10.1016/j.celrep.2020.107573.
40. Gou, L.T.; Kang, J.Y.; Dai, P.; Wang, X.; Li, F.; Zhao, S.; Zhang, M.; Hua, M.M.; Lu, Y.; Zhu, Y.; et al. Ubiquitination-Deficient Mutations in Human Piwi Cause Male Infertility by Impairing Histone-to-Protamine Exchange during Spermiogenesis. *Cell* **2017**, *169*, 1090-1104 e1013, doi:10.1016/j.cell.2017.04.034.
41. Zhao, S.; Gou, L.-T.; Zhang, M.; Zu, L.-D.; Hua, M.-M.; Hua, Y.; Shi, H.-J.; Li, Y.; Li, J.; Li, D.; et al. piRNA-Triggered MIWI Ubiquitination and Removal by APC/C in Late Spermatogenesis. *Developmental Cell* **2013**, *24*, 13-25, doi:10.1016/j.devcel.2012.12.006.
42. Alshaer, W.; Zureigat, H.; Al Karaki, A.; Al-Kadash, A.; Gharaibeh, L.; Hatmal, M.M.; Aljabali, A.A.A.; Awidi, A. siRNA: Mechanism of action, challenges, and therapeutic approaches. *Eur J Pharmacol* **2021**, *905*, 174178, doi:10.1016/j.ejphar.2021.174178.
43. Cecere, G. Small RNAs in epigenetic inheritance: from mechanisms to trait transmission. *FEBS Letters* **2021**, *595*, 2953-2977, doi:10.1002/1873-3468.14210.
44. Tatiparti, K.; Sau, S.; Kashaw, S.; Iyer, A. siRNA Delivery Strategies: A Comprehensive Review of Recent Developments. *Nanomaterials* **2017**, *7*, doi:10.3390/nano7040077.
45. Seroussi, U.; Lugowski, A.; Wadi, L.; Lao, R.X.; Willis, A.R.; Zhao, W.; Sundby, A.E.; Charlesworth, A.G.; Reinke, A.W.; Claycomb, J.M. A comprehensive survey of C. elegans argonaute proteins reveals organism-wide gene regulatory networks and functions. *Elife* **2023**, *12*, doi:10.7554/eLife.83853.
46. Tam, O.H.; Aravin, A.A.; Stein, P.; Girard, A.; Murchison, E.P.; Cheloufi, S.; Hodges, E.; Anger, M.; Sachidanandam, R.; Schultz, R.M.; et al. Pseudogene-derived small interfering RNAs regulate gene expression in mouse oocytes. *Nature* **2008**, *453*, 534-538, doi:10.1038/nature06904.
47. Watanabe, T.; Totoki, Y.; Toyoda, A.; Kaneda, M.; Kuramochi-Miyagawa, S.; Obata, Y.; Chiba, H.; Kohara, Y.; Kono, T.; Nakano, T.; et al. Endogenous siRNAs from naturally formed dsRNAs regulate transcripts in mouse oocytes. *Nature* **2008**, *453*, 539-543, doi:10.1038/nature06908.

 doi:10.20944/preprints202506.1663.v1

48. Phillips, C.M.; Almeida, M.V.; de Jesus Domingues, A.M.; Ketting, R.F. Maternal and zygotic gene regulatory effects of endogenous RNAi pathways. *PLOS Genetics* **2019**, *15*, doi:10.1371/journal.pgen.1007784.
49. Ha, M.; Kim, V.N. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol* **2014**, *15*, 509-524, doi:10.1038/nrm3838.
50. Pasquinelli, A.E.; Reinhart, B.J.; Slack, F.; Martindale, M.Q.; Kuroda, M.I.; Maller, B.; Hayward, D.C.; Ball, E.E.; Degnan, B.; Muller, P.; et al. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature* **2000**, *408*, 86-89, doi:10.1038/35040556.
51. Czech, B.; Hannon, G.J. Small RNA sorting: matchmaking for Argonautes. *Nature Reviews Genetics* **2010**, *12*, 19-31, doi:10.1038/nrg2916.
52. Das, P.P.; Bagijn, M.P.; Goldstein, L.D.; Woolford, J.R.; Lehrbach, N.J.; Sapetschnig, A.; Buhecha, H.R.; Gilchrist, M.J.; Howe, K.L.; Stark, R.; et al. Piwi and piRNAs Act Upstream of an Endogenous siRNA Pathway to Suppress Tc3 Transposon Mobility in the Caenorhabditis elegans Germline. *Molecular Cell* **2008**, *31*, 79-90, doi:10.1016/j.molcel.2008.06.003.
53. Champroux, A.; Tang, Y.; Dickson, D.A.; Meng, A.; Harrington, A.; Liaw, L.; Marzi, M.; Nicassio, F.; Schlaeger, T.M.; Feig, L.A. Transmission of reduced levels of miR-34/449 from sperm to preimplantation embryos is a key step in the transgenerational epigenetic inheritance of the effects of paternal chronic social instability stress. *Epigenetics* **2024**, *19*, 2346694, doi:10.1080/15592294.2024.2346694.
54. Chen, T.-H.; Chen, J.-A. Multifaceted roles of microRNAs: From motor neuron generation in embryos to degeneration in spinal muscular atrophy. *eLife* **2019**, *8*, doi:10.7554/eLife.50848.
55. Benito, E.; Kerimoglu, C.; Ramachandran, B.; Pena-Centeno, T.; Jain, G.; Stilling, R.M.; Islam, M.R.; Capece, V.; Zhou, Q.; Edbauer, D.; et al. RNA-Dependent Intergenerational Inheritance of Enhanced Synaptic Plasticity after Environmental Enrichment. *Cell Reports* **2018**, *23*, 546-554, doi:10.1016/j.celrep.2018.03.059.
56. Huang, Z.H.; Du, Y.P.; Wen, J.T.; Lu, B.F.; Zhao, Y. snoRNAs: functions and mechanisms in biological processes, and roles in tumor pathophysiology. *Cell Death Discov* **2022**, *8*, 259, doi:10.1038/s41420-022-01056-8.
57. Bortolin, M.L.; Ganot, P.; Kiss, T. Elements essential for accumulation and function of small nucleolar RNAs directing site-specific pseudouridylation of ribosomal RNAs. *EMBO J* **1999**, *18*, 457-469, doi:10.1093/emboj/18.2.457.
58. Bergstrand, S.; O'Brien, E.M.; Coucoravas, C.; Hrossova, D.; Peirasmaki, D.; Schmidli, S.; Dhanjal, S.; Pederiva, C.; Siggens, L.; Mortusewicz, O.; et al. Small Cajal body-associated RNA 2 (scaRNA2) regulates DNA repair pathway choice by inhibiting DNA-PK. *Nature Communications* **2022**, *13*, doi:10.1038/s41467-022-28646-5.
59. Rogelj, B.; Božič, J.; Bratkovič, T. Functional diversity of small nucleolar RNAs. *Nucleic Acids Research* **2020**, *48*, 1627-1651, doi:10.1093/nar/gkz1140.
60. Jin, G.; Xu, M.; Zou, M.; Duan, S. The Processing, Gene Regulation, Biological Functions, and Clinical Relevance of N4-Acetylcytidine on RNA: A Systematic Review. *Molecular Therapy - Nucleic Acids* **2020**, *20*, 13-24, doi:10.1016/j.omtn.2020.01.037.
61. Philippe Ganot, M.-L.B., and Tama´ s Kiss. Site-Specific Pseudouridine Formation in Preribosomal RNA Is Guided by Small Nucleolar RNAs. **1997**.
62. Zong, T.; Yang, Y.; Zhao, H.; Li, L.; Liu, M.; Fu, X.; Tang, G.; Zhou, H.; Aung, L.H.H.; Li, P.; et al. tsRNAs: Novel small molecules from cell function and regulatory mechanism to therapeutic targets. *Cell Prolif* **2021**, *54*, e12977, doi:10.1111/cpr.12977.
63. Tao, E.W.; Cheng, W.Y.; Li, W.L.; Yu, J.; Gao, Q.Y. tiRNAs: A novel class of small noncoding RNAs that helps cells respond to stressors and plays roles in cancer progression. *Journal of Cellular Physiology* **2019**, *235*, 683-690, doi:10.1002/jcp.29057.
64. Elkordy, A.; Mishima, E.; Niizuma, K.; Akiyama, Y.; Fujimura, M.; Tominaga, T.; Abe, T. Stress-induced tRNA cleavage and tiRNA generation in rat neuronal PC12 cells. *Journal of Neurochemistry* **2018**, *146*, 560-569, doi:10.1111/jnc.14321.

 doi:10.20944/preprints202506.1663.v1

65. Canan Kuscu, P.K., MANJARI KIRAN, ZHANGLI SU, ASRAR MALIK, and ANINDYA DUTTA. tRNA fragments (tRFs) guide Ago to regulate gene expression post-transcriptionally in a Dicer-independent manner. **2018**, doi:10.1261/rna.066126
66. 10.1261/rna.066126.called.
67. Burton, A.C.; Beirne, C.; Gaynor, K.M.; Sun, C.; Granados, A.; Allen, M.L.; Alston, J.M.; Alvarenga, G.C.; Calderon, F.S.A.; Amir, Z.; et al. Mammal responses to global changes in human activity vary by trophic group and landscape. *Nat Ecol Evol* **2024**, *8*, 924-935, doi:10.1038/s41559-024-02363-2.
68. Brembs, B.; Kikusui, T.; Nakanishi, K.; Nakagawa, R.; Nagasawa, M.; Mogi, K.; Okanoya, K. Cross Fostering Experiments Suggest That Mice Songs Are Innate. *PLoS ONE* **2011**, *6*, doi:10.1371/journal.pone.0017721.
69. Latorre-Pellicer, A.; Moreno-Loshuertos, R.; Lechuga-Vieco, A.V.; Sánchez-Cabo, F.; Torroja, C.; Acín-Pérez, R.; Calvo, E.; Aix, E.; González-Guerra, A.; Logan, A.; et al. Mitochondrial and nuclear DNA matching shapes metabolism and healthy ageing. *Nature* **2016**, *535*, 561-565, doi:10.1038/nature18618.
70. Wu, L.L.; Russell, D.L.; Wong, S.L.; Chen, M.; Tsai, T.-S.; St John, J.C.; Norman, R.J.; Febbraio, M.A.; Carroll, J.; Robker, R.L. Mitochondrial dysfunction in oocytes of obese mothers: transmission to offspring and reversal by pharmacological endoplasmic reticulum stress inhibitors. *Development* **2015**, *142*, 681-691, doi:10.1242/dev.114850.
71. Arai, J.A.; Li, S.; Hartley, D.M.; Feig, L.A. Transgenerational Rescue of a Genetic Defect in Long-Term Potentiation and Memory Formation by Juvenile Enrichment. *The Journal of Neuroscience* **2009**, *29*, 1496-1502, doi:10.1523/jneurosci.5057-08.2009.
72. Curley, J.P. Social enrichment during postnatal development induces transgenerational effects on emotional and reproductive behavior in mice. *Frontiers in Behavioral Neuroscience* **2009**, *3*, doi:10.3389/neuro.08.025.2009.
73. Gapp, K.; Bohacek, J.; Grossmann, J.; Brunner, A.M.; Manuella, F.; Nanni, P.; Mansuy, I.M. Potential of Environmental Enrichment to Prevent Transgenerational Effects of Paternal Trauma. *Neuropsychopharmacology* **2016**, *41*, 2749-2758, doi:10.1038/npp.2016.87.
74. McGreevy, K.R.; Tezanos, P.; Ferreiro-Villar, I.; Palle, A.; Moreno-Serrano, M.; Esteve-Codina, A.; Lamas-Toranzo, I.; Bermejo-Alvarez, P.; Fernandez-Punzano, J.; Martin-Montalvo, A.; et al. Intergenerational transmission of the positive effects of physical exercise on brain and cognition. *Proc Natl Acad Sci U S A* **2019**, *116*, 10103-10112, doi:10.1073/pnas.1816781116.
75. Cintado, E.; Tezanos, P.; De Las Casas, M.; Muela, P.; McGreevy, K.R.; Fontan-Lozano, A.; Sacristan-Horcajada, E.; Pignatelli, J.; de Ceballos, M.L.; Del Hierro, M.J.; et al. Grandfathers-to-Grandsons Transgenerational Transmission of Exercise Positive Effects on Cognitive Performance. *J Neurosci* **2024**, *44*, doi:10.1523/JNEUROSCI.2061-23.2024.
76. Grayson, B.; Leger, M.; Piercy, C.; Adamson, L.; Harte, M.; Neill, J.C. Assessment of disease-related cognitive impairments using the novel object recognition (NOR) task in rodents. *Behav Brain Res* **2015**, *285*, 176-193, doi:10.1016/j.bbr.2014.10.025.
77. Takahashi, Y.; Morales Valencia, M.; Yu, Y.; Ouchi, Y.; Takahashi, K.; Shokhirev, M.N.; Lande, K.; Williams, A.E.; Fresia, C.; Kurita, M.; et al. Transgenerational inheritance of acquired epigenetic signatures at CpG islands in mice. *Cell* **2023**, *186*, 715-731.e719, doi:10.1016/j.cell.2022.12.047.
78. Benatti, R.O.; Melo, A.M.; Borges, F.O.; Ignacio-Souza, L.M.; Simino, L.A.P.; Milanski, M.; Velloso, L.A.; Torsoni, M.A.; Torsoni, A.S. Maternal high-fat diet consumption modulates hepatic lipid metabolism and microRNA-122 (miR-122) and microRNA-370 (miR-370) expression in offspring. *British Journal of Nutrition* **2014**, *111*, 2112-2122, doi:10.1017/s0007114514000579.
79. Wilson, R.A.; Deasy, W.; Hayes, A.; Cooke, M.B. High fat diet and associated changes in the expression of micro-RNAsin tissue: Lessons learned from animal studies. *Molecular Nutrition & Food Research* **2017**, *61*, doi:10.1002/mnfr.201600943.
80. tZhao, X.; Chen, Z.; Zhou, Z.; Li, Y.; Wang, Y.; Zhou, Z.; Lu, H.; Sun, C.; Chu, X. High-throughput sequencing of small RNAs and analysis of differentially expressed microRNAs associated with high-fat diet-induced hepatic insulin resistance in mice. *Genes & Nutrition* **2019**, *14*, doi:10.1186/s12263-019-0630-1.
81. Zheng, X.; Li, Z.; Wang, G.; Wang, H.; Zhou, Y.; Zhao, X.; Cheng, C.Y.; Qiao, Y.; Sun, F. Sperm epigenetic alterations contribute to inter- and transgenerational effects of paternal exposure to long-term

 doi:10.20944/preprints202506.1663.v1

psychological stress via evading offspring embryonic reprogramming. *Cell Discovery* **2021**, *7*, doi:10.1038/s41421-021-00343-5.

82. Yin, X.; Anwar, A.; Wang, Y.; Hu, H.; Liang, G.; Zhang, C. Paternal environmental exposure-induced spermatozoal small noncoding RNA alteration meditates the intergenerational epigenetic inheritance of multiple diseases. *Front Med* **2022**, *16*, 176-184, doi:10.1007/s11684-021-0885-y.
83. Wang, Y.; Chen, Z.P.; Hu, H.; Lei, J.; Zhou, Z.; Yao, B.; Chen, L.; Liang, G.; Zhan, S.; Zhu, X.; et al. Sperm microRNAs confer depression susceptibility to offspring. *Sci Adv* **2021**, *7*, doi:10.1126/sciadv.abd7605.
84. Skinner, M.K.; Ben Maamar, M.; Sadler-Riggleman, I.; Beck, D.; Nilsson, E.; McBirney, M.; Klukovich, R.; Xie, Y.; Tang, C.; Yan, W. Alterations in sperm DNA methylation, non-coding RNA and histone retention associate with DDT-induced epigenetic transgenerational inheritance of disease. *Epigenetics & Chromatin* **2018**, *11*, doi:10.1186/s13072-018-0178-0.
85. Wei, X.; Zhang, Z.; Gu, Y.; Zhang, R.; Huang, J.; Li, F.; He, Y.; Lu, S.; Wu, Y.; Zeng, W.; et al. Inter- and trans-generational impacts of real-world PM(2.5) exposure on male-specific primary hypogonadism. *Cell Discov* **2024**, *10*, 44, doi:10.1038/s41421-024-00657-0.
86. Zeng, L.; Zhou, J.; Zhang, Y.; Wang, X.; Wang, M.; Su, P. Differential Expression Profiles and Potential Intergenerational Functions of tRNA-Derived Small RNAs in Mice After Cadmium Exposure. *Front Cell Dev Biol* **2021**, *9*, 791784, doi:10.3389/fcell.2021.791784.
87. Yeshurun, S.; Hannan, A.J. Transgenerational epigenetic influences of paternal environmental exposures on brain function and predisposition to psychiatric disorders. *Molecular Psychiatry* **2018**, *24*, 536-548, doi:10.1038/s41380-018-0039-z.
88. Martos, S.N.; Tang, W.Y.; Wang, Z. Elusive inheritance: Transgenerational effects and epigenetic inheritance in human environmental disease. *Prog Biophys Mol Biol* **2015**, *118*, 44-54, doi:10.1016/j.pbiomolbio.2015.02.011.
89. Lumey, L.H.; Stein, A.D.; Susser, E. Prenatal famine and adult health. *Annu Rev Public Health* **2011**, *32*, 237-262, doi:10.1146/annurev-publhealth-031210-101230.
90. Pembrey, M.; Saffery, R.; Bygren, L.O. Human transgenerational responses to early-life experience: potential impact on development, health and biomedical research. *Journal of Medical Genetics* **2014**, *51*, 563-572, doi:10.1136/jmedgenet-2014-102577.
91. Monaco, A.P. An epigenetic, transgenerational model of increased mental health disorders in children, adolescents and young adults. *Eur J Hum Genet* **2021**, *29*, 387-395, doi:10.1038/s41431-020-00726-4.
92. Locasale, J.W.; Nätt, D.; Kugelberg, U.; Casas, E.; Nedstrand, E.; Zalavary, S.; Henriksson, P.; Nijm, C.; Jäderquist, J.; Sandborg, J.; et al. Human sperm displays rapid responses to diet. *PLOS Biology* **2019**, *17*, doi:10.1371/journal.pbio.3000559.
93. Gaspari, L.; Haouzi, D.; Gennetier, A.; Granes, G.; Soler, A.; Sultan, C.; Paris, F.; Hamamah, S. Transgenerational Transmission of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Effects in Human Granulosa Cells: The Role of MicroRNAs. *Int J Mol Sci* **2024**, *25*, doi:10.3390/ijms25021144.
94. Marczylo, E.L.; Amoako, A.A.; Konje, J.C.; Gant, T.W.; Marczylo, T.H. Smoking induces differential miRNA expression in human spermatozoa: a potential transgenerational epigenetic concern? *Epigenetics* **2012**, *7*, 432-439, doi:10.4161/epi.19794.
95. Dickson, D.A.; Paulus, J.K.; Mensah, V.; Lem, J.; Saavedra-Rodriguez, L.; Gentry, A.; Pagidas, K.; Feig, L.A. Reduced levels of miRNAs 449 and 34 in sperm of mice and men exposed to early life stress. *Translational Psychiatry* **2018**, *8*, doi:10.1038/s41398-018-0146-2.
96. Oliva Trejo, J.A.; Tanida, I.; Suzuki, C.; Kakuta, S.; Tada, N.; Uchiyama, Y. Characterization of starvation-induced autophagy in cerebellar Purkinje cells of pHluorin-mKate2-human LC3B transgenic mice. *Scientific Reports* **2020**, *10*, doi:10.1038/s41598-020-66370-6.
97. Huypens, P.; Sass, S.; Wu, M.; Dyckhoff, D.; Tschop, M.; Theis, F.; Marschall, S.; Hrabe de Angelis, M.; Beckers, J. Epigenetic germline inheritance of diet-induced obesity and insulin resistance. *Nat Genet* **2016**, *48*, 497-499, doi:10.1038/ng.3527.
98. Fullston, T.; Palmer, N.O.; Owens, J.A.; Mitchell, M.; Bakos, H.W.; Lane, M. Diet-induced paternal obesity in the absence of diabetes diminishes the reproductive health of two subsequent generations of mice. *Hum Reprod* **2012**, *27*, 1391-1400, doi:10.1093/humrep/des030.

 doi:10.20944/preprints202506.1663.v1

99. Crisóstomo, L.; Jarak, I.; Rato, L.P.; Raposo, J.F.; Batterham, R.L.; Oliveira, P.F.; Alves, M.G. Inheritable testicular metabolic memory of high-fat diet causes transgenerational sperm defects in mice. *Scientific Reports* **2021**, *11*, doi:10.1038/s41598-021-88981-3.
100. Haberman, M.; Menashe, T.; Cohen, N.; Kisliouk, T.; Yadid, T.; Marco, A.; Meiri, N.; Weller, A. Paternal high-fat diet affects weight and DNA methylation of their offspring. *Scientific Reports* **2024**, *14*, doi:10.1038/s41598-024-70438-y.
101. Rodgers, A.B.; Morgan, C.P.; Bronson, S.L.; Revello, S.; Bale, T.L. Paternal Stress Exposure Alters Sperm MicroRNA Content and Reprograms Offspring HPA Stress Axis Regulation. *The Journal of Neuroscience* **2013**, *33*, 9003-9012, doi:10.1523/jneurosci.0914-13.2013.
102. Gapp, K.; van Steenwyk, G.; Germain, P.L.; Matsushima, W.; Rudolph, K.L.M.; Manuella, F.; Roszkowski, M.; Vernaz, G.; Ghosh, T.; Pelczar, P.; et al. Alterations in sperm long RNA contribute to the epigenetic inheritance of the effects of postnatal trauma. *Molecular Psychiatry* **2018**, *25*, 2162-2174, doi:10.1038/s41380-018-0271-6.
103. Klosin, A.; Casas, E.; Hidalgo-Carcedo, C.; Vavouri, T.; Lehner, B. Transgenerational transmission of environmental information in C. elegans. *Science* **2017**, *356*, 320-323, doi:10.1126/science.aah6412.
104. Dias, B.G.; Ressler, K.J. Parental olfactory experience influences behavior and neural structure in subsequent generations. *Nature Neuroscience* **2013**, *17*, 89-96, doi:10.1038/nn.3594.
105. tNakamura, N.; Yoshida, N.; Suwa, T. Three major reasons why transgenerational effects of radiation are difficult to detect in humans. *Int J Radiat Biol* **2024**, *100*, 1297-1311, doi:10.1080/09553002.2023.2187478.
106. Sharma, A.; Singh, P. Detection of transgenerational spermatogenic inheritance of adult male acquired CNS gene expression characteristics using a Drosophila systems model. *PLoS One* **2009**, *4*, e5763, doi:10.1371/journal.pone.0005763.
107. Shi, J.; Zhang, Y.; Tan, D.; Zhang, X.; Yan, M.; Zhang, Y.; Franklin, R.; Shahbazi, M.; Mackinlay, K.; Liu, S.; et al. PANDORA-seq expands the repertoire of regulatory small RNAs by overcoming RNA modifications. *Nat Cell Biol* **2021**, *23*, 424-436, doi:10.1038/s41556-021-00652-7.
108. Guérin, T.M.; Palladino, F.; Robert, V.J. Transgenerational functions of small RNA pathways in controlling gene expression inC. elegans. *Epigenetics* **2013**, *9*, 37-44, doi:10.4161/epi.26795.
109. Ow, M.C.; Hall, S.E. Inheritance of Stress Responses via Small Non-Coding RNAs in Invertebrates and Mammals. *Epigenomes* **2023**, *8*, doi:10.3390/epigenomes8010001.
110. Al Jowf, G.I.; Snijders, C.; Rutten, B.P.F.; de Nijs, L.; Eijssen, L.M.T. The Molecular Biology of Susceptibility to Post-Traumatic Stress Disorder: Highlights of Epigenetics and Epigenomics. *International Journal of Molecular Sciences* **2021**, *22*, doi:10.3390/ijms221910743.
111. Perez, M.F.; Lehner, B. Intergenerational and transgenerational epigenetic inheritance in animals. *Nature Cell Biology* **2019**, *21*, 143-151, doi:10.1038/s41556-018-0242-9.
112. Sharma, A. Transgenerational epigenetics: Integrating soma to germline communication with gametic inheritance. *Mech Ageing Dev* **2017**, *163*, 15-22, doi:10.1016/j.mad.2016.12.015.
113. Daxinger, L.; Whitelaw, E. Understanding transgenerational epigenetic inheritance via the gametes in mammals. *Nature Reviews Genetics* **2012**, *13*, 153-162, doi:10.1038/nrg3188.