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Dad's legacy: Epigenetic reprogramming and paternal inflammatory memory in offspring health

Shamila D. Alipoor[1], Parisa Norouzitallab[2], Anita Öst[1] & Maria Lerm[1]

From the [1]Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden; [2]Department of Applied Animal Science and Welfare, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden

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Over the past decade, numerous reports have highlighted intergenerational and even transgenerational epigenetic effects resulting from parental exposure to diets, toxins, and stress. In many cases, these parentally induced phenotypes do not seem to confer an obvious benefit, making it challenging to understand the evolutionary drivers behind them. In this perspective, we discuss recent observations in humans and rodents indicating that a parental infection or vaccination can enhance the offspring's ability to cope with infections. Such parental priming of their offspring's immune system and cellular defense would provide immediate protection to the newborn, offering a clear evolutionary advantage. Here, focusing mainly on paternal effects, we propose that a parentally induced inflammatory memory in the offspring could be the underlying mechanism for many of the reported inter- and transgenerational effects. Sperm-borne RNA could be a triggering signal to initiate inflammatory pathways in early embryogenesis. This gene-regulatory state would then be maintained via epigenetic mechanisms throughout each mitosis and last for the individual's lifetime. The accumulating understanding that diet, stress, toxins, and infections affect offspring health raises important questions about public health policies. There is an urgent need to understand what consequences different exposures during sensitive time windows have on future generations.

Keywords: epigenetic, immunity, intergenerational, molecular genetics, sncRNA

Anita Öst and Maria Lerm contributed equally.

Epigenetic reprogramming, trained immunity, and inflammatory memory

Trained immunity is today a well-established concept in immunobiology, adding to the repertoire of [1] described adaptive responses to infection in higher organisms (Fig. 1). Epigenetic changes associated with trained immunity equip innate immune cells with the capacity to mount a stronger response at a second exposure to a pathogen. Bacillus Calmette–Guérin (BCG) is a live attenuated strain of *Mycobacterium bovis* that was developed in the early 20th century as a vaccine to fight the tuberculosis pandemic. In the years after the introduction of BCG in Europe, Swedish physician Carl Naeslund observed that childhood mortality was strongly reduced in BCG-vaccinated cohorts [1] (reviewed in [2]). Importantly, this protection was not limited to a reduction in cases of tuberculosis but included reduced mortality by other infections [2]. This was the first documented finding of heterologous immunity induced by a vaccine, and more recently, the finding has been reproduced in a number of cohort studies in Africa. The emerging picture is that vaccination with live vaccines—such as the BCG [3], measles [4], and polio [5] vaccines—influences and rewires immunity in a way that is not explained by the development of specific antibodies and T cells. Instead, the underlying mechanism seems to rely on epigenetic reprogramming to generate cellular subsets or phenotypes tailored toward the control and elimination of different pathogens [6]. In addition to immune cells, skin epithelial stem cells, tissue resident macrophages, muscle stem cells, nasal epithelial stem cells, pancreatic epithelia, and Schwann cells

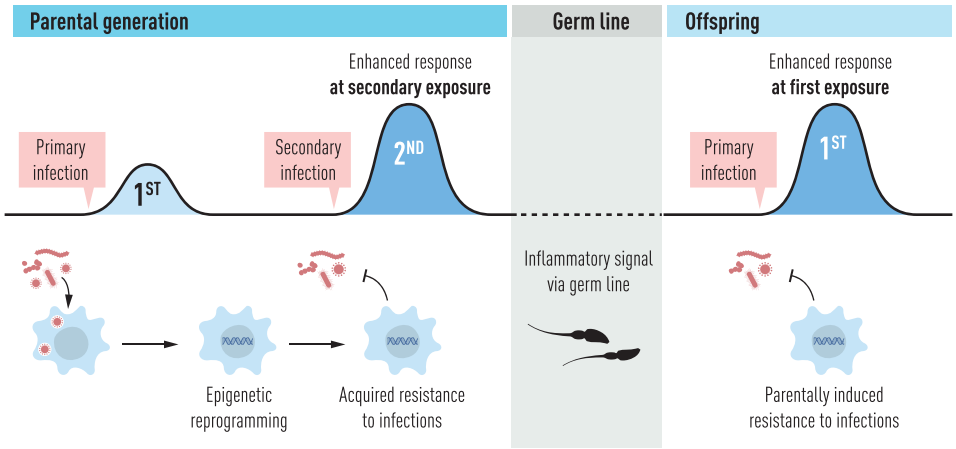

Fig. 1 *Model for parentally induced inflammatory memory. In the parental generation, a primary infection enhances responses to subsequent challenges through epigenetic reprogramming of cells with immune- or barrier function. Paternal signals such as sperm-borne RNA can induce inflammatory pathways during early embryogenesis, which thereafter are maintained via epigenetic mechanisms. The resulting resilience against infection provides an evolutionary advantage in conditions with persisting infection pressure, whereas it may predispose for inflammation-driven disease.*

also retain a memory of inflammation [7]. A more general term—encompassing different phenotypes of trained immunity and effects in non-immune cells—is therefore "inflammatory memory".

There are a handful of papers supporting that both the father and mother can prime their offspring for an environment requiring resistance to infections (Fig. 1). A Danish team has done extensive research on how BCG vaccine protects not only children but also their future grandchildren [8, 9]. One study was conducted on a cohort in Bissau, Guinea-Bissau, as part of the Bandim Health Project, which operates a Health and Demographic Surveillance System across six urban districts in the area. Interestingly, BCG vaccination in mothers had the most pronounced effect, whereas fathers vaccination also contributed to improved survival in infants and young children, suggesting intergenerational transmission of the heterologous protection conferred by BCG [10]. These findings highlight inherited health benefits of BCG vaccination and the importance of further research into intergenerational effects of inflammatory memory that inform vaccination policies. In a study from Norway, parental tuberculosis could be linked to offspring asthma [11]. A significantly increased risk of developing asthma was observed in children whose parents had TB in their childhood or youth, but the effect was more pronounced if the parents had TB during childhood and was strongest in the mother–daughter line [11]. Whether the hyperresponsiveness of airways associated with asthma is mechanistically linked to protection against TB disease remains to be explored. Another Norwegian study showed intergenerational effects on helminth infection with *Toxocara canis* and offspring allergy, with the strongest impact from father to daughter and mother to son [12]. Allergies involve the activation of eosinophils, whose main function is to fight multicellular parasites. This connection suggests that functional assays for anti-parasite responses should be included in the assessment of intergenerational epigenetic changes predisposing for asthma.

It was recently reported by Katzmarski et al. that mice had an increased immune response and higher survival rates when infected with *Candida albicans* if their fathers had been exposed to the same pathogen [13]. This transmitted trained immunity response also increased the offspring's resistance to bacterial pathogens such as *Escherichia coli*. In a similar study, Kaufmann et al. could not reproduce the findings [14]. The reason for the disparate conclusions is unclear, but as discussed later, the developmental window for the exposure might be important. Katzmarski et al. exposed their male mice to an infection when they were 6-week old (which corresponds to early puberty) [13]. In the study by Kaufmann et al., the age of the founder males at induction of trained immunity is not clearly defined [14]. In both studies, 1×10^4 CFU of *C. albicans* was

applied at F0 generation to train the immune system of the mice. However, the F1 generation challenge doses varied significantly, with Katzmarski et al. using 1×10^7 CFU and Kaufmann et al. using 1×10^5 CFU. This difference in challenge dose, along with developmental stage variation, may have influenced the variation in phenotypic outcome observed between the two studies.

Another study looked at the transgenerational impact of infection by the protozoan parasite *Toxoplasma gondii* [15], one of the most widespread zoonotic pathogens and which is known to alter the behavior of infected rodents, making them easy prey for cats. This is a consequence of parasite penetration into the rodent brain, thereby abrogating the natural fear of cats and allowing the parasite to complete its life cycle in the feline gut [16]. In that study, 6–8-week old mice were infected with the parasite and mated with uninfected females after a month on antibiotics to eliminate the infection [15]. The F1 and F2 offspring were assessed for behavioral changes, revealing altered stress responses and partial impairment of cognitive functions remaining throughout the F2 generation. Analysis of F0 sperm small RNA species revealed substantial changes to tRNA, micro-RNA (miRNA), and piwi-interacting RNA (piRNA) species, mapping to genes involved in immunity, reproduction, and metabolism. Importantly, microinjection of small RNA isolated from F0 sperm into naïve oocytes recapitulated the effects observed in the breeding experiment [15]. Further, it was shown that males and females exhibited differences in their cognitive impairment. This sexual dimorphism in paternal inheritance is a well-documented phenomenon in rodents that significantly influences disease susceptibility, manifestation, and outcomes.

Offspring phenotypes resulting from paternal exposure to non-infection-related stressors

It has been suggested that trained immunity and metabolic disease share common roots, and that trained immunity, in part, could explain the epidemiological link between infections and cardiovascular disease [17]. In line with this, a Western type of diet (similar to that used to induce paternal intergenerational responses) induced trained immunity in atherosclerosis-prone Ldlr^{-}/$^{-}$ mice [18]. In addition, inflammation induced by repeated injections of lipopolysaccharide resulted in metabolic disorder phenotypes such as glucose intolerance and obesity in the next generation. This phenotype was mediated through the angiogenin pathway [19]. These results indicate a shared etiology between trained immunity and metabolic disease even across generations. Furthermore, it has been shown that chronic stress primes innate immune responses in both mice and humans [20]. Before moving on to discuss potential mechanisms for intergenerational transmission of immune memory, we will therefore review what is known from studies on human and model organisms regarding non-infection-related exposures such as diet, toxins, and stress. Because the mother has a large impact on the developing fetus over a long time, many mechanistic studies have focused on the more limited contribution from the paternal line. Therefore, this perspective will discuss these aspects and will also provide suggestions on how today's knowledge gap can be addressed. Table S1 summarizes the evidence of paternal transmission of primed phenotypes in human datasets and animal models as discussed in the following sections.

Impact of food availability

By using historical records, including harvests and food prices from Överkalix—an geographically isolated community in northern Sweden—Pembrey et al. discovered that food abundance during the slow growth period (SGP) for boys (aged 9–12) negatively correlates with longevity of the grandchildren [21]. Notably, the impact differed by gender; the food supply of the paternal grandfather correlated with the mortality risk ratio of grandsons, whereas the paternal grandmother's food supply was associated solely with granddaughters' mortality risk ratios [21, 22]. Subsequently, the Uppsala Birth Cohort Multigeneration Study—a substantially larger cohort—replicated the Överkalix findings. It validated the influence of paternal grandfathers' prepubertal food supply on grandchildren's mortality risk yet failed to establish a synergistic connection between the food supply of paternal grandmothers and their descendants' mortality [23, 24]. The reasons behind the sensitivity of the SGP for boys remain unknown, but it is worth noting that it coincides with a pivotal proliferative phase of cells in the reproductive system.

The study of paternal dietary effects in mice was pioneered by Jimenez-Chillaron [25] and Carone [26]. These initial reports have been followed by over 20 studies, which have employed various dietary regimens and revealed diverse phenotypic outcomes in the offspring (recently reviewed in [27]). Many of these studies have used a chronic dietary challenge either in utero or from postweaning (prepuberty). There is, however, a recent study that looks at the dynamics of a diet-intervention [28]. In this study, mice were challenged with a high-fat diet for 2 weeks, followed by a control diet for 4 weeks to investigate whether the diet-intervention leads to long-lasting intergenerational effects or if it is transient. The study found that the effects of paternal diets are rapid but transient, as after a 4-week period on a control diet following the intervention, no intergenerational metabolic effects were observed. This aligns with previous human studies, which demonstrated that dietary changes can rapidly affect the profile of small non-coding RNAs (sncRNA) in sperm, but these changes may not persist long-term [29, 30]. It is therefore likely that there are several and possibly unrelated, diet-induced pathways that can change the offspring phenotype. Based on the existing observations, paternal diet significantly impacts the health status of progenies through multiple pathways, influencing metabolic programming. Historical and experimental evidence highlights the role of prepubertal nutrition and dietary interventions in shaping offspring phenotypes, with effects that can be transient or lasting. These findings emphasize the sensitivity of key developmental periods and the potential of paternal nutrition as a modifiable factor in reducing disease risk across generations.

Impact of exposure to toxic substances

Two large European cohort studies, RHINESSA and ECRHS, investigated the impact of tobacco smoke on respiratory health in three-four generations. Both studies found that the association of smoking in fathers to asthma in offspring only was significant if the fathers were smoking during their SGP [31, 32]. Importantly and surprisingly, with reference to direct effects of passive smoking, there was no correlation between fathers' smoking habits as adults and the occurrence of asthma in their children [31]. Prepubertal smoking of fathers has also been shown to correlate with increased body fat in their sons [33]. More recently, the findings have been corroborated by the identification of a strong DNA methylation signature mapping to smoking- and asthma-related pathways [34]. The study—which compared prepubertal and preconception smoking in fathers in the RHINESSA and the Avon Longitudinal Study of Parents and Children cohorts—clearly demonstrated that the DNA methylation changes were found in prepubertal smoking and not if fathers' smoking onset was after puberty [34]. In a study by Vallaster et al. on nicotine exposure of prepubertal male mice, xenobiotic signals increased the resistance of offspring to nicotine and cocaine, possibly through enhancing their liver metabolism [35]. In another mouse study, nicotine exposure in postpubertal founder males was found to influence the behavioral phenotypes across several generations and correlate with DNA methylation changes in founder males' sperm [36]. A recent study on smoking delete - nicotine and cigarettes in war veterans found epigenetic changes in the gene encoding aryl hydrocarbon receptor (AHR) repressor [37], which is one of the genes that display clearly altered DNA methylation in response to smoking [38]. The AHR system has evolved over 600 million years as a mechanism to handle toxic hydrocarbons [39]. Interestingly, epigenetic regulation of the AHR system has been linked to airway hyperresponsiveness and the pathogenesis of asthma [40], thus providing a possible mechanistic link between smoking and offspring predisposition for asthma.

Like in many animal studies on environmental toxins such as pesticides, plasticizers, and dioxins (reviewed in [41]), Thorson et al. exposed pregnant female rats to a pulse treatment of a toxic compound at the onset of fetal gonadal sex determination. Then, the impact of the exposure on male F1, F2, and F3 generations was monitored with respect to reproductive health, metabolism, and ageing-related diseases [42]. Such studies have provided strong evidence for transgenerational epigenetic adverse effects of toxic substances in a number of species (reviewed in [43]). A recent systematic review compiled the evidence for epigenetic consequences of mammalian male exposure to endocrine-disrupting compounds (EDC, including plasticizers, pesticides, and polycyclic aromatic hydrocarbons) presented in numerous studies [44]. The studies collectively found evidence for EDC-induced puberty- or adult-onset of diseases and epigenetic alterations in DNA methylation patterns that were propagated across generations originating from exposed founder males [45]. Exposure of 4-week-old CD-1 (ICR) male mice to bisphenol A (BPA) could disrupt spermatogenesis,

reduce sperm count and motility, and alter DNA methylation patterns in spermatozoa, with significant effects observed in the F0–F2 generations and partially in F3. These changes were linked to multigenerational impact on oxidative stress, reduced ATP levels, and damage to testicular tissue. The findings highlight BPA's reproductive toxicity and suggest potential risks for human fertility through occupational or environmental exposure [46].

Traumatic stress experiences

The study of multigenerational effects of paternal trauma has gained significant attention in epigenetics and behavioral neuroscience. Paternal experiences—particularly traumatic events—can leave a legacy that extends beyond the individual, influencing the biology and behavior of offspring across generations. Research in humans and mice has provided compelling evidence for these phenomena, often linked to epigenetic modifications and stress-response mechanisms [44, 47].

Dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis is a well-documented contributor to psychiatric disorders, yet the precise mechanisms behind this phenomenon remain unclear. The glucocorticoid receptor (GR)—a nuclear receptor protein critical for HPA axis feedback—modulates key physiological processes such as stress response, metabolism, immune function, and development. Decreased GR expression—often linked to epigenetic modifications—is a hallmark of impaired HPA axis regulation. Studies have consistently demonstrated higher DNA methylation levels in the hippocampal GR 1F promoter among adult suicide victims with histories of childhood abuse, suggesting that this epigenetic alteration disrupts GR expression and contributes to long-term mental health vulnerabilities [48–50]. Additionally, recent research has revealed a strong correlation between decreased GR expression and altered gene expression in the prefrontal cortex (PFC) of teenage suicide victims who suffered from major depression, substance abuse, or conduct disorders [51]. These findings indicate that early developmental trauma—especially from primary caregivers—alters neurobiological systems by epigenetically modulating the expression of key regulatory genes, such as the GR.

Subsequent studies identified higher GR promoter methylation in circulating leukocytes of adults exposed to childhood maltreatment [52]. Further investigations on GR pathways demonstrated that the GR pathways can also control stress response across generations. Yehuda et al. conducted one of the pioneering studies in this field where the team revealed that offspring of fathers with PTSD exhibited higher GR-1F promoter methylation in the absence of maternal PTSD, whereas offspring of both parents with PTSD showed lower methylation levels [53]. Clustering analysis confirmed distinct effects of maternal and paternal PTSD on clinical outcomes, indicating differential mechanisms in the intergenerational transmission of trauma. These observations highlight the complexity of trauma's epigenetic and psychological legacy, with clustering of clinical measures revealing distinct profiles associated with maternal and paternal PTSD, suggesting gender-specific mechanisms in the intergenerational transmission of trauma-related effects.

In another study, it was shown that paternal childhood trauma—such as physical, emotional, and sexual abuse—is linked to DNA methylation changes in sperm, potentially affecting offspring development. This study identified significant methylation differences in genes such as MAPT and CLU (neuronal function), PRDM16 (fat cell regulation), and SDK1 (immune function). These findings suggest an epigenetic pathway through which childhood abuse could impact future generations [54].

In a study conducted by Zheng et al., long-term psychological stress (restraining) in male mice was shown to contribute to the transgenerational inheritance of phenotypic disorders, including adverse impacts on reproductive, behavioral, and metabolic pathways [55]. DNA methylation profiling of sperm and embryo samples of three consecutive generations showed transgenerational inheritance of differentially methylated regions in DNA.

Gapp et al. report transgenerational transmission of behavioral and metabolic phenotypes in a mouse model of paternal postnatal trauma. The study found that early postnatal stress induces neurochemical alterations in the PFC of founder males that then transmit the signal across the paternal line to modify neuronal metabolic responses to acute stress [56]. In another mouse study that examined potential adaptive consequences of early life trauma, Gapp et al. demonstrated that

both female and male offspring of founder males exposed to postnatal traumatic stress displayed better goal-directed strategies (e.g., adaptation to new conditions) [57]. The results from these studies in mammals underline the importance of an adequate study design—for example, timing of paternal exposure and architecture of the offspring environment—to elucidate possible benefits of a modified epigenome.

Examining potential mechanisms for transmission of environmental signals across the body of an organism

Before we move on to discuss the possible mechanisms of how signals from stressful exposures such as infections can be transmitted to future generations, we will summarize the current knowledge on how an epigenetically encoded memory of previous exposure to microbial components can be established in the body of an organism. We will explore the possibility of direct interaction of stem cells with microbial components as well as indirectly via stem cell uptake of exosomes released by infected cells carrying instructions for epigenetic rewiring.

The molecular mechanism of a long-term memory in trained immunity—direct exposure of progenitor cells?

Monocytes and macrophages that make up an important first line of defense are terminally differentiated and are relatively short-lived [58]. Therefore, direct establishment of the memory in these cells is less likely to be attributed to propagation and maintenance of the memory for a long period of time [6]. Several studies have shown that pivotal to the establishment of trained immunity is the participation of hematopoietic stem cells (HSCs), residing within the bone marrow (BM). BCG vaccination, for example, exerts a multifaceted influence on HSCs, culminating in enhanced differentiation and a shift in the epigenomic landscape as demonstrated both in humans and mice [58]. Although BCG has not been proven to directly infect HSCs, it has been found in the BM, and its presence impacts the expansion of HSC progenitors [58, 59]. This reprogramming entails a remarkable enhancement of gene expression associated with myelopoiesis, encompassing key cytokines such as interferon-gamma (IFN-γ), tumor necrosis factor-α, and interleukin-1β, all of which are central for eliciting protective immune responses against mycobacterial threats [58]. Importantly, transplanting BM from BCG-vaccinated mice into naïve mice was sufficient to confer enhanced immunity against *Mycobacterium tuberculosis* through transferred trained HSCs [60]. The direct exposure of HSCs demonstrates that the BCG-induced immune phenotype depends on viable BCG and that it persists in the absence of its triggers. It does not explain the molecular events that are involved in the establishment of trained immunity in other non-hematopoietic cell types.

Molecular mechanisms of a long-term memory in trained immunity—exosomes and noncoding RNA

As a variety of RNA species have been linked to epigenetic rewiring, exosomes are attractive as potential vehicles of RNA signals from cells directly exposed to a pathogen or stressor to distal cells and tissues. Exosomes are evolutionarily conserved lipid bilayer nanovesicles [61], which can serve as conduits for cell-to-cell communication, including transfer of miRNA, tsRNA, rsRNA, long noncoding RNAs (lncRNA), and piRNA that modulate epigenetic profiles in recipient cells [62]. Epi-miRNAs—a subset of miRNAs—target key epigenetic regulators such as DNMT and histone deacetylases, inducing changes to recipient cells' epigenetic makeup [63]. When confronted with stress, somatic cells within tissues release exosomes that can reach both the BM and the reproductive organs, including the epididymis, allowing transmission to gamete cells, thereby linking lifestyle to the sperm epigenome [64, 65]. Although the role of exosomes as vehicles for transmission of RNA signals across the body is well-established, their involvement in conveying instructions for induction of trained immunity remains elusive.

"Immune gene priming lncRNAs" (IPLs) have been demonstrated to deposit epigenetic marks onto promoters of immune-related genes and induce trained immunity [66]. IPLs link extracellular signals with epigenetic regulatory enzymes, effectively directing them to their intended target gene promoters. Illustratively, the archetype of this mechanism is upstream master lncRNA of the inflammatory chemokine locus, which proficiently primes chemokine promoters [66]. β-Glucan, a classical inducer of trained immunity, triggers a nuclear factor of activated T cells-mediated increase in the transcription of several IPLs [66]. Further enriching our understanding is the contribution of NeST (*Nettoie Salmonella pas Theiler's*) RNA, another lncRNA associated with the trained immunity paradigm [67]. NeST RNA governs the epigenetic modifications of the IFN-γ locus, thereby amplifying its expression and bolstering responses

against intracellular bacterial pathogens such as *Salmonella* [67].

These mechanisms underscore the interconnectedness of ncRNAs, exosomes, and epigenetic regulation in shaping adaptive responses and transmitting environmental signals across tissues and possibly also across generations.

Examining potential mechanisms for transmission of environmental signals across the paternal line to the next generation

Passing paternal environmental information to the next generation

Paternally induced inter- or transgenerational effects are sometimes referred to as "epigenetic inheritance." The problem is that the term 'epigenetic' has several definitions but, most often, it is used as a synonym to DNA-methylation or histone-modifications. The concept of epigenetic inheritance therefore implies the utilization of an epigenetic mechanism such as DNA methylation, histone modification, and noncoding RNA to maintain a memory over one or several generations [68–73]. Consequently, it is expected that such a mechanism would be very similar to Mendelian inheritance, as an epigenetic modification on a specific locus will be remembered across generations. There have been many attempts to prove this hypothesis, but with some exceptions [55, 74, 75], environmentally induced changes in F0 sperm have not been detected in the same genomic loci in F1 somatic tissues, or F1–F2 sperm [26, 76].

Other observations that argue against an inheritance of epigenetic marks in mammals are (i) the massive erasure of epigenetic marks at the beginning of embryogenesis, (ii) reports that the phenotype sometimes skips a generation [77], and (iii) the heterogeneity of the offspring phenotype. The variability of offspring phenotypes, induced by parental environmental exposures, can be explained by the occurrence of metastable epialleles. Metastable epialleles are identical alleles that are variably expressed due to epigenetic modifications established very early in development. These epigenetic modifications are then mitotically inherited and maintained into adulthood. In the Agouti (Avy) model, frequently cited as an example of mammalian epigenetic inheritance, the variable coat color is determined by the metastable epiallele Avy. The coat color ranges from yellow to brown, with all shades in between. The maternal, but not paternal, coat color phenotype influences the offspring's coat color distribution. Offspring of yellow mothers are more likely to have yellow coats compared to those of brown-coated mothers, but yellow mothers can also have offspring with darker coats. Thus, the maternal phenotype shifts the probability of the offspring's coat color, rather than determining it in a strictly Mendelian manner. Given the expected fitness benefits arising from heterogeneity among offspring in a changing environment, including a varying repertoire of circulating infections, mechanisms that promote such heterogeneity may be evolutionarily advantageous (Fig. 2).

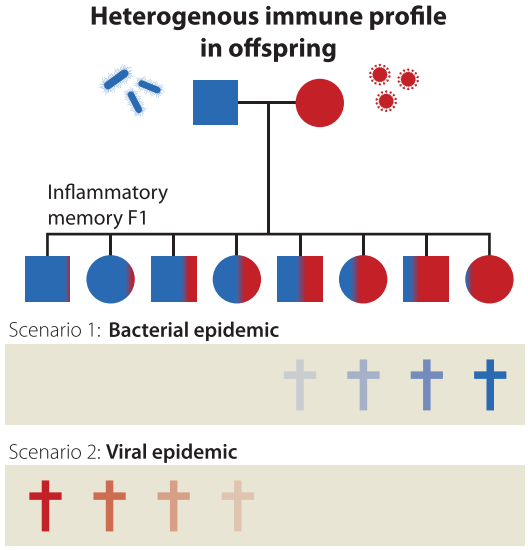

Fig. 2 *Hypothetical pedigree diagram of epigenetic traits linked to inflammatory memory reflecting the heterogeneity expected in studies of parentally induced phenotypes. Offspring with heterogeneous immune profiles may have survival advantages against certain pathogens (i.e., bacteria vs. viruses).*

As a complement to the concepts of epigenetic inheritance, we would like to draw an analogy with the emerging field of trained immunity and other manifestations of inflammatory memory. It can be projected that the stem cells in the early zygote in a similar way as macrophages, epithelial cells, and BM stem cells, retain a long-term memory of a priming inflammatory signal delivered by the sperm, thereby inducing an inflammatory memory state in all, or most, of the cells in the offspring (Fig. 3). It would also pose an example of a trade-off situation because a hyperactive inflammatory response would increase the risk for

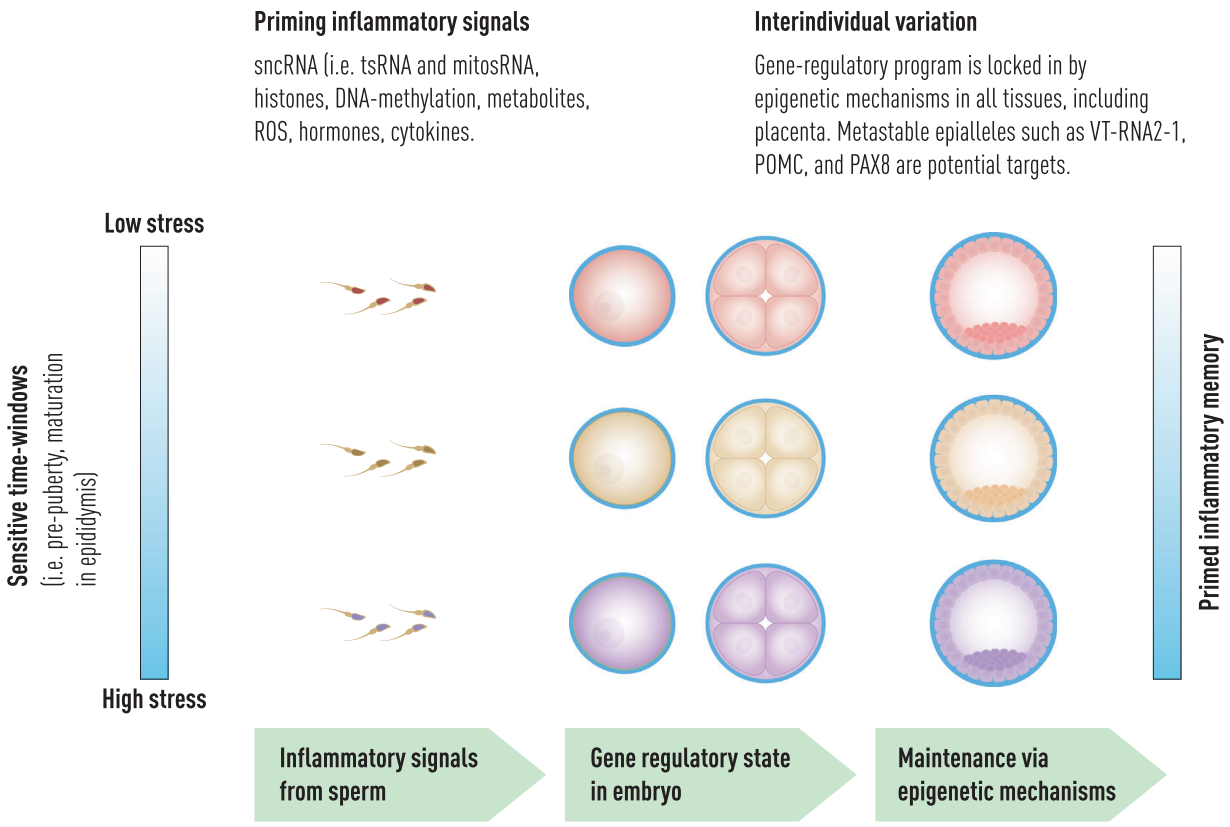

Fig. 3 *During sensitive time-windows, male gamete development is susceptible to environmental stressors. Diverse stressors such as diet, toxins, and infections change the load of sperm-borne small noncoding RNA (sncRNA) and other potent signaling molecules. At fertilization, the egg responds to inflammatory signals in sperm by activation of immune-regulatory genes. Establishment of stable epigenetic states may occur during early cell divisions and placental development to create a unified epigenetic profile in offspring traits, including immune cell activity.*

inflammatory diseases, including obesity, asthma, psoriasis, cancer, and cardiovascular disease in adults. Notably, several putative metastable epialleles in humans—such as VT-RNA2-1, POMC, and PAX8—are involved in the regulation of the immune system (reviewed in [78]). Because the variable gene expression of these metastable epialleles is established early in development, likely before gastrulation, they are potential targets for an initiating signal from sperm. A priming signal in sperm that sets the epigenetic state of immunity-related metastable epialleles could, similar to coat color in Avy mice, shift the phenotypic distribution to a more or less primed state. However, unlike the visible coat color in Avy mice, this difference in a primed-state phenotype might go unnoticed until challenged by an infection, high-fat diet, or chronic stress. In a scenario with infection pressure, this concept translates to the hypothetical model described in Fig. 2. For example, parental exposure to viral infections may rewire some offsprings' immunity toward resilience against viral infection, whereas some offspring retain better resilience against bacteria.

The generation of a paternal environmentally induced signal

Spermatogonial stem cells (SSCs)—located at the basement membrane of the seminiferous tubules within the testes—are responsible for replenishing sperm in adult males [79]. From an inflammatory memory perspective, it is conceivable that an epigenetically controlled long-term memory of inflammation in SSCs could persist through spermatogenesis, influencing the characteristics and priming signals of sperm. Alternatively, support cells—such as Sertoli cells in the testes and cells

in the epididymis—might retain long-term memories of infections, inflammation, or other stressors. However, it is important to consider the time window during which such long-lived memories can be established. Research suggests that an epigenetically encoded inflammatory memory of previous exposure to microbial components can manifest at any developmental stage or age [80]. In contrast, paternal intergenerational responses are sensitive to specific developmental windows, such as during the SGP (Fig. 3). What makes the developmental period unique? Could the prepubertal maturation of the male reproductive tract make its cells more susceptible to environmental influences, as proposed [70, 71, 81]? During the SGP, both SSCs and their epithelial support cells, Sertoli cells, undergo transient phases of proliferation before committing to their differentiated states [82]. Once differentiated, Sertoli cells form junctions that create the blood–testis barrier, effectively isolating the testis from the bloodstream. Additionally, these cells acquire immunoregulatory functions and begin expressing genes related to immunity [83].

Notably, the metabolic phenotype of prepubertal human spermatogonia differs from that of adult spermatogonia. SSC development relies on distinct metabolic transitions, shifting from oxidative phosphorylation to anaerobic metabolism [84]. Metabolites serve as both substrates for DNA methylation and histone modifications and as regulators of epigenetic enzymes. Therefore, any stress-induced metabolic imbalances during this critical period could significantly influence the development of epigenetically mediated effects on sperm, impacting both the current and subsequent generations.

In epididymis, maturing sperm are directly or indirectly exposed to hormones, metabolites, and epididymosomes loaded with different RNA species and proteins, for example, transcription factors (Fig. 3). Hypothetically, this response could be intergenerational, that is, affect the F1 generation but not transmitted to and beyond F2. Seminal fluid is mainly produced in the prostate gland and in the seminal vesicle. These structures are relatively exposed to blood components, which potentially permits exposure to environmental cues. Exosomes, hormones, and other factors in seminal fluid may influence the zygote, embryo, and/or the environment in the uterus, thereby conveying epigenetic information through F2 [85].

Potential molecular mechanisms for a paternally induced primed phenotype

Sperm DNA-methylation, retained histones, RNA, and transcription factors have all been suggested to be mediators of paternally transmitted intergenerational effects, and this has been extensively reviewed elsewhere [70, 86, 87].

From the perspective of inflammatory memory, we would like to highlight an emerging theme that could unify paternal intergenerational responses and priming of the immune response. Over the past decades, advances in sncRNA-seq have revealed that sperm contains a complex mix of sncRNAs. These range from well-characterized miRNAs to semi-understood piRNAs, tsRNAs, and rsRNAs, as well as less understood small RNAs derived from the mitochondrial genome, Y-RNA, and Vault-RNA (vtRNA) [72]. Notably, the sncRNAs found in sperm are very similar to those found in exosomes. Specifically, Y-RNA- and vtRNA-derived small RNAs—present in both sperm and exosomes—have been suggested to regulate the innate immune system. Although the innate immune system has evolved to sense foreign nucleic acids from invading pathogens, some endogenous RNAs—including Y-RNA and vtRNA—can also serve as substrates for classic nucleic acid sensors [88–91].

Vaults are large ribonucleoprotein complexes, roughly three times the size of a ribosome, with a molecular weight of about 13 MDa. These complexes contain one or more vRNAs or vtRNAs ranging from 86 to 141 bases in length. One of the four described vtRNAs—vtRNA2-1—is, as discussed above, a putative metastable epiallele in humans [78]. Both global and myeloid-specific knockouts of the major vault protein gene have been shown to worsen high-fat diet-induced obesity and insulin resistance in mice via the NF-κB signaling pathway. This effect was accompanied by increased macrophage infiltration and heightened peripheral inflammatory responses [92].

vtRNAs are pivotal in the innate immune system, particularly in virus–host interactions [91]. VtRNAs are recognized by pattern recognition receptors such as RIG-I and PKR. This recognition

is crucial for the immune system to distinguish between self and nonself nucleic acids, initiating antiviral responses.

In the context of viral infections, vtRNAs activate antiviral pathways, including the PKR and OAS/RNase L systems. These pathways inhibit viral replication and promote the degradation of viral RNA, thereby limiting the spread of the virus within the host. The processing of vtRNAs into smaller fragments further enhances their regulatory roles in these antiviral mechanisms.

Alterations in vtRNA expression can significantly impact the host's ability to respond to viral infections, linking these changes to various diseases, including cancer and autoimmune disorders. The methylation status of vtRNAs also plays a role in their stability and processing, affecting their function in virus–host interactions.

Paternal control of the placenta

If an inflammatory memory is induced in the early zygote, it means that all cells—including the trophoblasts that are essential for the placenta—would have the same priming (Fig. 3). BCG-trained innate immunity in female mice leads to fetal growth restriction by altering immune cell profile in the mouse developing placenta [93]. Similarly, periconceptional exposure of a male mouse to DDT leads to reduced offspring birth weight, placental size, and glycogen levels, with more pronounced effects in male progeny placentas [94]. In this study, male progeny exhibits severe glucose intolerance, whereas females show slightly improved glucose handling—highlighting sexual dimorphism in metabolic function. In another recent study, Argaw-Denboba et al. demonstrated that preconception perturbation of the gut microbiome in mouse fathers caused a reduction in birth weight of the offspring [95]. The study provides a mechanistic link to the observed placental insufficiency via altered metabolite and RNA profiles in the male reproductive tract and proposes a gut-germline axis controlling offspring phenotype. Of note, the study found the phenotype to be reversible through restoration of the paternal microbiome [95]. The findings align with epidemiological evidence linking environmental impact of the parental exposure on fetal growth impairments and suggest sperm epigenomic changes as a potential mechanism. Future studies are needed to further elucidate the male contribution to placental phenotype and its consequences for the offspring. In summary, we highlight the possible windows of opportunity that may define the route of transmission of trained immunity and other epigenetic traits from father to offspring. Although there is substantial evidence of the existence of such transmission, there is a scarcity in cellular and molecular evidence of how such transmission occurs. With the ambition to inspire future research studies to provide such evidence, we are suggesting consideration of the aspects listed in Table 1. Careful experimental design may help increase the likelihood of establishing a robust scientific foundation for this expanding research area.

Possible future clinical implications

The emerging picture is that not only maternal, but also paternal, preconceptional health influences the future health of their children. This raises important questions for couples planning to have a baby and for public health policies. For example, Svanes' findings indicate that boys who smoke in the prepubertal period may later in life father children with increased risk for asthma [96]. If verified in independent cohorts, these findings should be communicated to the public. Furthermore, it is vital to begin systematically collecting data to evaluate inter- or transgenerational effects of diet, stress, toxins, and infections and also commonly used medications such as corticosteroids [97]. It took mankind some time to recognize the harmful effect of DDT on the reproductive system of birds of prey, and similarly, recognizing patterns of inter- or transgenerational effects of these factors in humans is challenging. To achieve this, we need to track parental exposures and offspring health outcomes over several generations.

In this review, we propose that the intergenerational and transgenerational effects of diet, stress, toxins, and infections converge on immune system activity. If valid, this suggests that individuals inherit a spectrum of immune modalities, ranging from heightened responsiveness to more subdued activity, with diverse immune characteristics shaped by these influences. Therapies aiming to modulate the reactivity of the immune system hold great promise to achieve a balance between enhancing protective immunity and minimizing the potential for detrimental inflammatory responses,

Table 1. *Suggestions for experimental design in studies of paternally induced inflammatory memory.*

1	Calculate power, taking an expected heterogenous offspring response as well as sex-differences into account
2	Perform exposures at defined and relevant time points of male reproductive tract development, e.g., in utero (bearing trimesters in mind)—prepuberty—puberty—adult
3	Consider assessment of whether the response is transient or permanent
4	Perform RNA and DNAm profiling experiments on relevant tissues, cell types and subfractions (e.g., placenta, epididymal epithelium and epididymosomes, sertoli cells, sperm, sperm mitochondria)
5	Consider including many possible variants of noncoding RNA (miRNA, lncRNA, piRNA, mitosRNA, vtRNA and fragments of tRNA, rRNA and lncRNA) in the analyses
6	Test the resilience of the offspring generations to relevant cues such as infections or food availability
7	Ensure analyses include higher level perspectives (e.g., on a functional, modular level rather than, for example, focusing on singular CpG sites or just one mRNA species).
8	Repeat the same approach through male F3 to monitor the persistence of the response and capture the possible 'skipping-one-generation' phenomenon

ultimately leading to improved health outcomes and quality of life for individuals with diverse immunological challenges [98].

As previously discussed, early life administration of the BCG vaccine accelerates neonatal immune system development and induces long-lasting immune reprogramming. These effects, combined with its role in reducing neonatal mortality by preventing severe infections, underscore its transformative potential in early life interventions. Future strategies could focus on optimizing dosing and integrating BCG into broader immunization programs, particularly in high-risk settings.

There are many potential targets for modulating the activity of the immune system. In epidermal stem cells, over 1000 inflammatory memory domains have been characterized [99]. After a priming inflammation, these domains retain a chromatin-state with a higher accessibility, allowing a greater response when exposed to a second inflammatory event. Targeting the epigenetic pathways that promote such sustained activation is a key strategy to dampen the reactivity and promote an anti-inflammatory state. Many epigenetic drugs are under clinical trial, mainly for cancer treatment [100], but some of them might find applications in immunotherapies. Alternatively, it might be possible to use metabolic switches as drug targets. Trained monocytes switch metabolism from oxidative phosphorylation to glycolysis, thereby promoting mevalonate biogenesis [101]. Mevalonate has been shown to activate IGF1-R/mTOR/glycolysis, thus activating a self-sustaining loop. Therefore, statins are promising anti-inflammatory drugs.

Conflict of interest statement

ML is the founder of PredictMe, a company that develops algorithms that estimate an individual's health based on epigenetic data. The other authors declare no conflicts of interest.

Data availability statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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Correspondence: Anita Öst, Department of Biomedical and Clinical Sciences, Linköping University, 581 85 Linköping, Sweden.
Email: anita.ost@liu.se

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