Transgenerational Epigenetics
A Window into Paternal Health Influences on Offspring

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KEYWORDS

• DNA methylation • Transgenerational inheritance • Histone modifications • RNA • Sperm • Paternal age effect

KEY POINTS

- Sperm epigenetics is modifiable over the lifespan of an individual and is impacted by lifestyle decisions, diet, and exposures.
- Sperm epigenetic alterations are capable of altering fertility and offspring phenotype.
- Epigenetics modifications in the paternal germ line has the potential to positively influence offspring phenotype and overall fitness.

INTRODUCTION

The sperm epigenome is deeply important because of its potential effects on intergenerational (1 generation) and transgenerational (2 or more generations) trait inheritance, fertility, and its role in embryonic development.

Over a 50-year period in the latter half of the 20th century, fertility rates decreased significantly. In fact, the average sperm count decrease by more than 41% over that time, and the average seminal volume decreased from 3.4 to 2.75 mL.[1] This trend has continued since then and has begun to raise serious concerns in the health industry. Many factors negatively influence semen quality, such as smoking, age, and obesity, and a somewhat recently explored and powerful force, aberrant sperm epigenetics, may play a role in the negative impact of these signals on sperm function, fertility, and offspring health.[2,3] Although not considered to be independently causative of these fertility deficits, many studies suggest that perturbed epigenetic profiles in sperm contribute to infertility phenotypes, poor embryo quality, and even offspring abnormalities.[4–6]

The reproductive impacts of various toxicants or exposures vary greatly between men and women. In contrast with females, and with some exceptions, males generally do not become entirely infertile when exposed to toxins or as a result of aging, but instead display decreased fertility. This means that they can potentially parent children at older ages or after various exposure types when females may not have this same capacity. This fact often causes clinicians to overlook male fertility as a small barrier to pregnancy and focus on the sometimes absolute barriers to pregnancy that can occur in females. Although justified based on pregnancy data, there are consequences in pursuing this focused approach to fertility care. When taking into consideration the fact that men remain competent to father children despite advanced age or extreme environmental exposures along with recently discovered evidence demonstrating that age and environmental influences can cause significant changes in the

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heritable sperm epigenome, these facts reason that men have a great capacity to pass on environmentally influenced characteristics. In short, the fact that a man's fertility is robust over time and after various exposures does not mean that their ability to father healthy children is constant or unaffected. In reality, the fact that men can father children at very old ages or after years of exposures makes men more likely to impact the offspring's health. As a result, it is important to define the impact of various modifiers and what those modifications are capable of inducing in the offspring.

Indeed, studies have shown that epigenetic inheritance can occur via sperm through ancestral exposures, having a proven impact on offspring phenotype.[7–13] These exposures (including toxins or metabolic variations) and subsequent inheritance patters challenge anti-Lamarckian dogma that assumes changes caused by random mutations accumulate over many generations that ultimately lead to a change in a population. Instead, these patterns look more like the inheritance of acquired traits that Lamarck described, and are handed down in distinct ways. The first is through DNA methylation alterations in the paternal genome. DNA methylation (the addition of a methyl group to cytosines in the genome) has the capacity to impact gene transcription through hypermethylation and or hypomethylation at gene promoters which inhibits or facilitates access of transcriptional machinery to the gene promoter.[14] This modification directly on the DNA acts almost like molecular memory that can be passed on to the offspring at certain locations throughout the genome. The second is through RNA and RNA fragments that are found in or on the mature sperm. One study demonstrated this process by inoculating normal oocytes with sperm RNA fragments from mice given a high-fat diet; the metabolic functions of the pups were impaired.[15]

The sperm epigenome also affects the developing embryo. One study showed that global increases in sperm DNA methylation were associated with higher rates of infertility.[16] Thus, a normal sperm epigenome in general is recognized as important to fecundity, whereas abnormal methylation in sperm DNA is associated with infertility and poor outcomes in the offspring. Moreover, when mouse sperm DNA was deliberately hypomethylated through inhibition of the DNA methyltransferase proteins, similar outcomes was seen.[17]

In all, although just one of many influences, the sperm epigenome plays an important part in male fertility and it also seems to be important in the inheritance of acquired traits. This article focuses on the data that support this assertion.

TRANSGENERATIONAL INHERITANCE

Multiple studies demonstrate that male preconception lifestyle decisions have the potential to impact their offspring, for better or worse. Multiple epidemiologic studies have shown deleterious impacts on the offspring after exposure to various chemicals, cigarette smoke, and advancing age in men, to name a few. These troubling findings need to be more thoroughly understood, although promising headway is being made to address the issue at hand.

The recent interest in transgenerational epigenetic inheritance has driven a great deal of research addressing the impact of multiple modifiers (environmental toxins, drugs, lifestyle decisions, diet, aging, etc) of sperm epigenetic signatures and the downstream impact of these changes. This effort has reveled some interesting patterns and potential mechanisms of nongenetic inheritance that seem to fall into 2 distinct categories, either programmatic or disruptive. Examples of programmatic changes to gametes during an individual's life span are those that have the potential to alter the offspring's phenotype in a manner that makes the offspring more competent to respond to some environmental condition. In contrast, disruptive mechanisms of epigenetic inheritance are those that result from exposure to various toxins, environmental pollutants, or the aging process that leads to perturbed offspring phenotypes. Interestingly, these disruptive modifications often result in common abnormalities in the offspring such as neuropsychiatric disease, increased cancer susceptibility, and so on, regardless of the type of insult (aging, cigarette smoke, etc).

The mechanism(s) that account for transgenerational or intergenerational inheritance of specific alterations is not known, although interesting findings are accumulating quickly in the literature. It is likely that the etiology of the inheritance patterns may be unique between programmatic and disruptive transgenerational and intergenerational inheritance. Despite our lack of knowledge regarding the specific patterns of inheritance, it is clear that this phenomenon exists and that it has a significant, but often subtle, impact on the offspring. This finding is particularly true when considering the population wide shifts in cultural or societal activities (changes in diet, stress levels, age at conception, etc).

TWO SUBCATEGORIES OF EPIGENETIC INHERITANCE: PROGRAMMED AND DISRUPTIVE

Some of the earliest examples of potential epigenetic inheritance (and specifically transgenerational

inheritance) occurred in Scandinavia. Life for residents of Overkalix, a rural town in far Northern Sweden, in the 1800s was, like most small communities, heavily reliant on crop success to feed the populous. Unfortunately, there was a great deal of variation in the early 1800s in crop success, with multiple famines followed by periods of overabundance. This change in nutrition over a short period of time for a relatively isolated population has provided some of the most important epidemiologic data to date establishing patterns of nongenetic inheritance and has given rise to the interest in epigenetics and transgenerational inheritance that we see today. In fact, a great deal of the most alarming data regarding transgenerational inheritance has arisen from large-scale epidemiologic studies that have shown primarily deleterious impacts on the offspring as a result of environmental exposures in fathers and even in grandfathers.

One of the earliest reports that came from the Overkalix dataset suggested that boys who were 9 to 12 years of age during times with surplus food supplies in Overkalix had grandsons with a decreased lifespans compared with controls.[18] Interestingly, these same individuals, the grandsons of paternal grandfathers who were exposed to food surpluses, had increased mortalities from metabolic (diabetes) and cardiovascular disease.[19] Importantly, although a response from the father to the son (intergenerational inheritance) was observed (even after correction for early social circumstances), the impact of paternal grandfather nutrition remained the most impactful influence on longevity in these individuals.[20]

With these initial Swedish famine studies as a backdrop, investigators began exploring the impact of certain epigenetic modifiers with a specific emphasis on sperm epigenetic modifications and their downstream impact. As described, it seem that, from the growing body of literature, the impacts on offspring or grand offspring will fall largely into 2 categories, either being disruptive in the offspring or programmatic/advantageous.

Disruptive Heritability Patterns

The data that suggest that some intergenerational or transgenerational inheritance patterns are disruptive come in multiple forms. Some of the earliest studies have been focused on epidemiologic data, but new studies using both animal models and humans exist as well. We briefly describe a few examples of disruptive inheritance patterns with aging, obesity and diet, and cigarette exposure in fathers. We discuss the impact of these signals on the gametes as well as on the offspring where data are available.

Studies involving aging and reproduction rarely focus on the male partner. Because female age causes such a striking and absolute barrier to pregnancy, physicians are often more concerned with female age than with male partner age. There is justification in the literature to take this approach, but new and emerging studies available to us now would argue that the male partner should not be ignored. In fact, there is ample evidence in the literature that, as a father advances in age, the likelihood of their offspring developing a neuropsychiatric disorder (autism, schizophrenia, bipolar disease) is significantly increased.[21–26] Not only does this seem to occur from father to son, but 1 recent study has even described an increased incidence of autism in the offspring of older paternal grandfathers (a true transgenerational inheritance pattern).[27] These epidemiologic data opened the door to multiple studies in human and animal models. Smith and colleagues[28] demonstrated in mice that a similar phenotype exists in the offspring sired by older males, namely, that there is a decrease in social and exploratory behaviors the offspring of older males. Our work in human has also shown epigenetic patterns in the sperm that are impacted by age. We identified more than 100 regions in human sperm that have altered DNA methylation patterns as a result of aging.[29] These patterns are particularly interesting when taking into account their enrichment at genomic loci containing genes that have been implicated in schizophrenia and bipolar disorder. Interestingly, these alterations are so consistent that they were used by our laboratory to construct a germline age calculator that can use sperm methylation data to predict an individual's age with a high degree of accuracy.[30] Despite these intriguing findings, there are no analyses in the offspring of these individuals to truly confirm that the methylation alterations could be playing a role in the transmission of this effect over multiple generations. This circumstance is due to the nature of such a study in humans. However, there is a study performed Milekic and colleagues[31] in mice that seems to confirm that such transmission is possible in the context of aging in mammals. Milekic's group confirmed in mice the data previously produced that suggested there is an increase in behavioral abnormalities consistent with common neuropsychiatric disorders in the offspring of older male mice. Not only this, but they were able to identify DNA methylation alterations in the sperm of older fathers, similar to those identified in our human work, while also confirming altered DNA methylation and gene expression in the offspring.

Studies regarding the impact of paternal diet and obesity on offspring phenotype are also available and provide interesting insight into these unique patterns of inheritance. In 1 study in rats, it was found that males exposed to a high -fat diet had offspring and grand offspring with metabolic disorders and decreased insulin sensitivity.[32] Another study has shown that obesity alone in adult male mice could result in altered sperm epigenetic profiles as well as offspring phenotypic changes that persisted over at least 2 generations. The offspring of these obese males had increased adiposity and altered metabolism, and were also obese.[33] In humans, obese men have been shown to have DNA methylation alterations at specific loci in the sperm. In 1 study, these alterations were seen at imprinted genes.[34] Another study in morbidly obese patients sought to understand the reversibility of these alterations with the intervention of bariatric surgery. These patients had significant alterations in their sperm methylome before surgery and almost immediately after surgery there was a dramatic reprogramming of the sperm epigenome.[34] Among the most interesting studies of the heritability of obesity-related alterations to the sperm epigenome and associations to offspring health in humans to date was work that screened newborns who were fathered by obese men. These offspring had alterations in DNA methylation at the IGF2 locus, suggesting that there is some downstream impact of paternal obesity.

Of additional interest to the community is smoking and its impact on sperm DNA methylation and offspring phenotypes and disease susceptibility. In humans, our group has identified distinct genomic loci that have significant DNA methylation alterations in smokers.[35] However, it was not determined what the downstream impact of these alterations may be. Importantly, previous data have demonstrated the preconception cigarette smoking in men is associated with a variety of health consequences in the offspring, including an increased risk of cancers, aneuploidies, and birth defects. One such study showed that the offspring of fathers who smoke (before conception), coupled with mothers who do not, have an increased chance of being diagnosed with a variety of childhood cancers, including cancers blood and nervous system tumors.[36] Very recently, a group in Cost Rica identified an increased risk of leukemia in the offspring of fathers who consumed cigarette smoke before conception.[37] Additional studies in human sperm have identified alterations to important chromatin structures in the sperm of smokers.[38] One interesting study showed that male mice exposed to nicotine fathered offspring that had altered behavioral phenotypes over 2 generations. Specifically, it was found that the offspring of males exposed to nicotine had a decreased capacity for learning and attention and increased locomotor activity.

Although these studies represent only a small portion of the data that are available regarding the potentially disruptive nature of transgenerational inheritance, they clearly demonstrate that what the father does actually matters. Preconception lifestyle decisions and exposure to various modifiers places the offspring at significantly increased risk of various abnormalities.

Programmatic Heritability Patterns

Although many studies have shown that the impacts of a father's lifestyle decisions and exposures can result in metabolic disorders and an increased risk of various diseases, it is clear that some signals carried from parent to offspring in a nongenetic fashion are of a different type. Some even seem to be beneficial to the offspring, as if the gamete had the capacity to inform the offspring of some environmental condition and prepare them to cope with it in a unique way. The mechanisms that underlie this process are poorly understood and quite controversial, but great strides are being made at a rapid pace.

Perhaps one of the most dramatic displays of what seems to be a programmed inheritance of acquired traits was displayed in a study published in 2014 in *Nature Neuroscience*.[39] The authors of this study wanted to explore the impact of fear conditioning in male mice and determine if this conditioning could somehow impact the offspring. The study involved the scent acetophenone (an aromatic ketone commonly used in perfumes) and an electric shock. In brief, male mice were exposed to the scent while simultaneously being exposed to a gentle shock on their foot pads. The animals soon became conditioned to the smell and associated it with the shock and were startled each time the odor was introduced, regardless of the presence of an associated shock. These F0 animals were humanely killed and the sperm that were extracted were either used to generate new offspring or to perform DNA methylation analysis. Intriguingly, the offspring of males exposed to the shock would respond similarly to the odor. Specifically, they would display the startled phenotype when exposed to acetophenone unlike the control group, whose fathers were not exposed to the scent and subsequent shock. Further, the offspring of exposed males also had a higher sensitivity to acetophenone (they were able to identify the scent at lower concentrations).

Taken together, the offspring of males conditioned to the acetophenone scent with associated shock were afraid of this scent when it was detected. The authors of the study attempted to explore the mechanism by which this environmental queue was passed on the offspring. It was found that there was significantly altered methylation in the sperm of the males exposed to acetophenone and shock at the odor receptor gene Olfr151 (the receptor known to bind acetophenone). The authors propose that this is the method by which signals are handed down to the offspring.

To highlight the unique nature of each inheritance pattern (and the fact that they all do not necessarily fit neatly into either the programmatic inheritance category or the disruptive inheritance category), it is important to site additional work performed on nicotine. We mentioned a study elsewhere in this article with largely negative consequences on the offspring. However, other authors have also assessed the intergenerational inheritance patterns associated with paternal nicotine exposure and have identified some interesting findings that suggest there may be some more programmatic like responses by the offspring. In a recent publication from Oliver Rando's laboratory at the University of Massachusetts, they describe how paternal exposure of nicotine induced protective responses (a decreased sensitivity) in the offspring.[40] They found that the offspring of male mice exposed to nicotine could survive high levels of nicotine exposure; in the most extreme case, the animals were able to survive toxic levels of nicotine injection. This finding suggests that the offspring of nicotine-exposed males were desensitized to the drug by a still unknown mechanism.

Although many more studies exist that demonstrate the impact of transgenerational inheritance, these studies highlight what is known in the field today. The 2 studies that seem to suggest that the sperm are able to orient the offspring to a specific environment are remarkable. This finding truly does fly in the face of traditional thinking regarding the inheritance of traits through Darwinian natural selection, but may offer potential new avenues of exploration that will impact many fields from evolution to medicine.

SUMMARY

It is essential to understand the consequences of our decisions, particularly when those consequences can affect others. It is only recently that we have learned about the impacts of a father's preconception lifestyle decisions and exposures on their offspring. Although there remains a great deal of work that must be done to fully understand the mechanisms that underlie the process and the full impact these mechanism will have on the offspring, we must to not ignore what has already been demonstrated. These patterns of nongenetic inheritance of acquired characteristics have fundamentally changed the way that we think about the inheritance of traits and, in the future, we may be able to identify patterns in the sperm that are predictive of outcomes in the offspring such that we can generate highly personalized approaches to reproductive care. In an older male who wishes to father a child, for example, we may be able to help offer a precise probability of him having a child with a specific neuropsychiatric disease as a result of the work that is being performed today. There is a great deal that still needs to be explored and determined, but the potential impact of this work in the clinic and in our understanding basic biological principles is very significant.

DISCLOSURE

M.M. Grover has nothing to report. T.G. Jenkins is a share holder in Inherent Biosciences (an epigenetics company) and Nanonc (a microfluidics company specializing in sperm isolation).

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