The most common vices of men can damage fertility and the health of the next generation

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Abstract

Animal and human studies demonstrate that acquired paternal traits can impair both a male's fertility and the health of his offspring, including advanced age, smoking, stress, trauma, under-nutrition, infection, toxin exposure, and obesity. Many of these factors lead to similar changes to neurological, behavioural, and/or metabolic functioning in offspring. The molecular mechanisms that both respond to the paternal environment and act to transmit traits to offspring are beginning to emerge. This review focuses on three vices of men (alcohol consumption, overweight/obesity, and tobacco smoking) that damage fertility and pose risks to offspring health. These vices are not only the three most prevalent but are also leading risk factors for death and disability adjusted life years (DALYs) worldwide. Moreover, given that these vices are predominantly self-inflicted, interventions aimed at mitigating their consequences are readily identified.

Key Words

- ▶ male fertility
- ▶ paternal programming
- ▶ sperm epigenetics
- ▶ environmental exposure

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Introduction

There is growing evidence from animal and human studies that demonstrate that acquired paternal traits can impair both a male's fertility and the health of his offspring, including advanced age, smoking, stress, trauma, under-nutrition, infection, toxin exposure and obesity. Curiously, many of these factors manifest as impaired neurological, behavioural and/or metabolic functioning in offspring. The underlying molecular mechanisms that respond to the paternal environment and act as vectors of intergenerational transmission are beginning to emerge. This review focuses on three vices of men (alcohol consumption, overweight/obesity and tobacco smoking) that damage fertility and pose risks to offspring health. These vices are not only the three most prevalent but are

also leading risk factors for death and disability adjusted life years (DALYs) worldwide.

Clearly, any epigenetic/genetic alterations induced by the paternal exposures responsible for transmission need to escape/bypass the substantial post-fertilisation reprogramming that occurs during embryo development. For example, paternal obesity alters the molecular composition of sperm, alters the developmental trajectory of resultant embryos and increases the incidence of obesity and metabolic disorders in offspring. Mechanistic candidates of paternal programming include changes to the sperm epigenome (e.g. DNA methylation, histone/protamine modifications, and sperm borne small non-coding RNAs), increased sperm DNA damage, aberrant

sperm DNA chromatin structure and components of seminal plasma. Understanding the molecular mechanisms underpinning paternal programming may lead to the development of interventions designed to reduce the disease burden in future generations, who were born to fathers exposed to these initiating factors. Given that these vices are predominantly self-inflicted, interventions aimed at mitigating their consequences are readily identified.

Paternal factors that impair sperm function or molecular composition and diminish offspring health

Overweight and obesity

Worldwide, more than 2.7 (~37.5%) billion people are either overweight (~2.1 billion, 28.4%) or obese (~0.7 billion, 9.1%), with ~2/3 of adults being overweight/obese in most westernised societies (Ng *et al.* 2014). As the prevalence of obesity is continually increasing so is its contribution to the burden of health, ranked 5th/10th as a risk factor for causing death/DALYs worldwide, respectively (http://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_full.pdf). Of concern, there is increasing evidence that paternal obesity in humans is not only associated with reduced fertility but also compromises the health of the next generation. For example, a study that spanned three generations of a Northern Sweden population (Överkalix), an excess of grandpaternal food was associated with reduced survivability and an increased risk of diabetes in their grandchildren (Kaati *et al.* 2002). Furthermore, an elevated paternal BMI is associated with increased BMI in his children, but in humans, this phenomenon cannot be separated from shared genetic predispositions and/or a common 'obesogenic' environment.

Animal models can circumvent many factors that confound human studies. Animal models of diet induced obesity that induce paternal programming have provided more direct evidence of impairment to the health of both first and second generation of offspring, manifesting as an increased risk of metabolic syndrome and subfertility (Fullston *et al.* 2013).

Smoking

A significant proportion of men worldwide to smoke tobacco, with ~22.7% of men at prime reproductive age continuing to smoke (<http://apps.who.int/gho/data/>

node.sdg.3-a-viz?lang=en) and smoking ranks as the 2nd/6th risk factor contributing to cause of death/DALYs globally, respectively (http://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_full.pdf). This is despite a broad understanding that cigarette smoke containing a vast array of toxic and mutagenic compounds, which besides damaging organs, also have deleterious effects on sperm. A recent meta-analysis of 5864 participants of 20 studies concluded that smoking is associated with reduced sperm count, motility and morphology with a magnified effect in moderate-to-heavy smokers compared to mild smokers (Sharma *et al.* 2016).

In addition, smoking also alters sperm microRNA content, greatly increases the abundance of reactive oxygen species (ROS) in the seminal plasma, increases sperm DNA fragmentation and increases oxidative DNA damage in sperm; as measured by 8-hydroxy-2'-deoxyguanosine (8-OHdG) lesions. Smokers are at increased risk of sperm defects such as partially or fully inactive mitochondria and non-intact acrosomes, in addition to changes to the proteome of their seminal plasma (Antoniassi *et al.* 2016). These changes to DNA and seminal plasma are hypothesised to further contribute to the increased childhood cancer risk seen in children born to male smokers, beyond the contribution of paternal smoking to the increased mutation load in both the father's germline and transmitted to his children (Linschooten *et al.* 2013).

Animal models replicate the findings from these human studies, whilst controlling for other paternal lifestyle factors. Smoking clearly has an impact on both male's fertility and the health of any children he fathers and likely acts through damage to the genome and epigenome of sperm.

Alcohol consumption

Surprisingly, given that >50% of men regularly drink alcohol (<http://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/drugusealcoholandsmoking/datasets/aduldrinkinghabits>), the impact of alcohol consumption on male fertility is not as well understood, and it ranks as the 8th/3rd worldwide risk factor as a cause of death/DALYs, respectively (http://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_full.pdf). Human studies are often confounded by the participants also being smokers and/or overweight/obese or not being controlled for the amount or duration of alcohol consumption. A recent study of 8344 men assessed the impact of alcohol consumption (via self-reported questionnaire) in the week

Figure 1

Overview of how the paternal environment might program offspring health as a result of three most common vices of men. The environment that a male is exposed to modulates the micro-environment within the testes/epididymis, which in turn alters the development/maturation of sperm – as a result sperm can harbour altered genetic/epigenetic material (increased DNA damage – oxidative an strand breaks and alterations to DNA methylation, small non-coding RNA content, and histone modifications) that is transferred to the oocyte at fertilisation. This subsequently alters the molecular makeup of the early embryo that leads to a delay in embryo/foetal development and the potential to impair offspring health.

prior to semen analysis on standard semen parameters and serum reproductive hormones, concluding that no correlation existed between alcohol consumption and any semen variable (count, motility or morphology). However, they reported a linear association between total alcohol consumption and total or free testosterone, possibly reflecting the impact that alcohol has on liver metabolism. It must be noted that the average self-reported alcohol consumption in this cohort was low to moderate (median of 8 units intake per week), and the study did not take into account other lifestyle factors or the alcohol consumption in the months prior (Jensen *et al.* 2014). Regardless, human cohort studies have associated paternal alcohol consumption with a range of neurological, learning, behavioural and growth anomalies in children (Finegersh *et al.* 2015).

Despite the lack of a consistent and reproducible offspring phenotype across the animal models of paternal alcohol exposure used to date, many of the phenotypes observed in children are recapitulated by animal models, and it also has been demonstrated to induce hypomethylation and oxidative stress in sperm (Finegersh *et al.* 2015).

Therefore, given the prevalence of paternal alcohol consumption further research is required to assess the impact of excessive, chronic or binge alcohol consumption on sperm genetics/epigenetics, semen quality, reproductive hormones, fertility and child health. Ideally, this would be undertaken in the absence of/or controlling for confounding factors to ensure that we understand the true impact of alcohol consumption on male fertility and child health.

Mechanisms of non-genetic paternal transmission to offspring

Prime candidate mechanisms that see paternal environmental cues passed to the next generation are non-genetic alterations within sperm such as small non-coding RNAs, DNA damage, DNA methylation and histone modifications. Although it must be noted that in the case of smoking, genetic damage in male gametes and offspring has been demonstrated (Linschooten *et al.* 2013) and any non-genetic transmission would likely act in concert to exacerbate genetic effects.

Sperm borne small non-coding RNAs

Mature sperm contain RNA, including a significant amount of small non-coding RNAs (sncRNAs), which are transferred to the oocyte upon fertilisation where they alter gene expression in the early embryo. One such subset of sncRNA is microRNAs, which are short, endogenous, single-stranded non-coding RNAs that can alter gene/protein expression by binding specific target sequences via either mRNA decay or translational repression. MicroRNAs may modulate other epigenetic regulators such as DNA methyltransferases and histone deacetylases and conversely be targets of epigenetic regulation themselves.

Sperm-borne microRNAs have been demonstrated to be important for embryo development as evidenced by embryonic arrest in embryos deficient of sperm derived microRNA, paternal microRNA-34c being critical for the first cleavage event and the microinjection of microRNA from 10 sperm resulting in embryonic lethality (Chen *et al.* 2016). The microinjection of multiple sncRNA or microRNA species are sufficient to recapitulate complex phenotypes including offspring behavioural and metabolic defects induced by a father's chronic stress (9 microRNAs), behavioural and metabolic defects induced by a father's early life trauma (entire sperm sncRNA content), or an offspring acquired metabolic disorder caused by a father's over-nutrition (entire sperm tsRNA fraction). The microinjection of 9 microRNAs changed in sperm by a father's chronic stress caused targeted degradation of stored maternal mRNAs and induced a cascade of molecular events in the early embryo that precede the adult offspring phenotype (Rodgers *et al.* 2015). Furthermore, the microinjection at supra-physiological concentrations of one microRNA into very early-stage embryos demonstrates that alterations

to embryonic abundance of a single microRNA can alter phenotypes in adult offspring, including metabolic syndrome caused the microinjection of microRNA-19b (Grandjean *et al.* 2015).

Although sperm microRNA content has been demonstrated to be altered by obesity and smoking it has not been demonstrated as a result of alcohol consumption to date, but alterations in microRNA profile have been observed in other cells types due to alcohol exposure (Finegersh *et al.* 2015). Therefore, the sncRNA content of sperm is capable of being modulated by the environment that a male is exposed to, alter the molecular makeup in the early embryo and induce phenotypes in resultant offspring; making them candidate agents for paternal programming.

Oxidative DNA damage

ROS can cause DNA strand breaks and oxidative damage to DNA (which can result in mutagenesis), peroxidation of unsaturated lipids and disruption to mitochondrial function. Sperm are highly susceptible to oxidative damage due to the lack of cytoplasmic scavenging enzymes and high concentrations of polyunsaturated fatty acids found in their plasma membranes. While physiological concentrations of ROS are vital for spermatogenesis and post ejaculation maturation, including capacitation and hyperactivation, these processes become impaired if the cells enter a state of oxidative stress (i.e. excessive ROS). Sperm DNA is partially protected from oxidative damage via the replacement of histones with protamines during spermatogenesis that form a terraform structure of tightly packed DNA. However, the protamination of sperm DNA is incomplete, with ~15% histone retention in human sperm. Therefore, while protamine bound DNA might be protected, the loci associated with retained histones remain more vulnerable to oxidative attack.

These histone-bound areas are not randomly distributed and are enriched at developmental important loci, including genes key to early embryo development. The 8-OHdG oxidative lesion, the most characterised in sperm, co-localises to the nuclear matrix and histone-bound DNA (Noblanc *et al.* 2013). Sperm cannot repair 8-OHdG lesions as they only contain the first enzyme in the base excision repair pathway (OGG1) and subsequently rely on the oocyte's enzymes required to complete this process after fertilisation (Smith *et al.* 2013), which if overwhelmed by the abundance of 8-OHdG lesions could theoretically lead to the incorporation of mismatched

bases and an increased mutation load in the resultant embryo/offspring.

An imbalance in ROS towards oxidative stress in sperm occurs as a result of the three most prevalent male exposures (male obesity, smoking and alcohol consumption), all of which also increase offspring susceptibility to disease, potentially in part via the mechanisms outlined above.

Other epigenetic mechanisms

The prime example of paternal influence on offspring phenotype via epigenetics is imprinting. Paternal imprinting disorders arise when the paternally inherited imprinted allele is expressed, at the expense of the maternal allele by mechanisms controlled by methylation, presumably transmitted via sperm DNA. How the three main vices of men alter sperm DNA methylation is not understood, but obesity is associated with hypomethylation of DNA from testes and late elongated spermatids (Fullston *et al.* 2013). Sperm DNA hypomethylation is also associated with alcohol exposure (Finegersh *et al.* 2015) and smoking (Kim *et al.* 2015). If this hypomethylation can persist into the embryo post-fertilisation it can alter the reprogramming of the male pronucleus in response to environmental exposures and lead to the onset of disease in offspring.

Although sperm protamines are replaced with maternal histones at fertilisation, paternal-bound histone segments are not replaced by the oocyte and therefore any modifications to these areas (i.e. from oxidative damage) are likely inherited unchanged into the embryo (Brykczynska *et al.* 2010). All three of these male exposures have also been demonstrated to alter histone modifications. Thus, sperm histone modifications due to these exposures hold the potential to alter gene expression in the early embryo and subsequently the growth of the developing embryo/foetus, ultimately effecting offspring health.

Notably, any casuistic transgenerational epigenetic signals must persist or be applied during two rounds of epigenetic reprogramming within the embryo/foetus. Epigenetic reprogramming ensures embryo totipotency and the incomplete removal of epi-mutations to prevent the transmission of disease to offspring. The epigenome of the pre-implantation embryo is incompletely reprogrammed during development, whereby DNA methylation is re-established (except for imprinted genes). Then, the primordial germ cells in the foetus undergo a second round of epigenetic reprogramming where

epigenetic marks are re-acquired in a sex, cell and tissue-specific manner that might lead to epigenetic inheritance into the next generation of offspring.

Concluding remarks

The most common vices of men influence sperm by creating genetic alterations, epigenetic marks and extracellular signals, which in turn affect offspring phenotype. Many non-genetic mechanisms might act in concert (and in conjunction with any genetic damage) as the agents of paternal transmission due to these exposures with the most widely recognised, but not limited to, alterations to sperm sncRNA (including microRNAs) content that modifies embryonic gene expression, oxidative damage to sperm DNA, methylation changes of (imprinted) genes maintained into the embryo, modifications (e.g. acetylation and methylation) of histones retained in sperm transmitted unaltered from sperm to embryo and seminal fluid composition influencing the response of the female reproductive tract prior to implantation. All these mechanisms are predicted to alter the molecular constitution of the developing embryo that subsequently induces pathologies in offspring. The study of the mechanisms potentially underpinning paternal programming identifies the pathways involved in the paternal transmission of disease to offspring (summarised in Fig. 1). Further investigation is required to understand how these molecular alterations result from the paternal exposure presumably during spermatogenesis and how reprogramming during embryo development is influenced, to lead to the onset of disease in offspring. Furthermore, relatively simple interventions (e.g. dietary intake, physical activity, cessation of alcohol/tobacco use) have demonstrated promising results for improving both sperm quality and offspring health. When fully understood, the non-genetic markers that cause paternal programming could be used to determine the potential programming load caused by paternal risk factors prior to transmission to offspring or as readouts of the effectiveness of any pre-conception interventions. Their mitigation might then ultimately act as a circuit breaker for the transgenerational transmission of obesity, thus improving the health and fertility of future generations.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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