

# The sperm factor: paternal impact beyond genes

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## **Abstract**

The fact that sperm carry more than just the paternal DNA has only been discovered just over a decade ago. With this discovery, the idea that the paternal condition may have direct implications for the fitness of the offspring had to be revisited. While this idea is still highly debated, empirical evidence for paternal effects is accumulating. Male condition not only affects male fertility but also offspring early development and performance later in life. Several factors have been identified as possible carriers of non-genetic information, but we still know little about their origin and function and even less about their causation. I consider four possible non-mutually exclusive adaptive and non-adaptive explanations for the existence of paternal effects in an evolutionary context. In addition, I provide a brief overview of the main non-genetic components found in sperm including DNA methylation, chromatin modifications, RNAs and proteins. I discuss their putative functions and present currently available examples for their role in transferring non-genetic information from the father to the offspring. Finally, I identify some of the most important open questions and present possible future research avenues.

35 **Introduction**

The importance of non-genetic factors for the transmission of information from parents to offspring is increasingly recognized (Bonduriansky and Day, 2009; Bonduriansky, 2012; Bonduriansky and Day, 2018). In animals, the relatively bigger size of the female gamete – the egg – and the resulting transfer of many different non-genetic components from the mother to her offspring has led to an early recognition of the role of maternal non-genetic effects in determining offspring phenotype (e.g. Dickerson, 1947; Willham, 1963; Legates, 1972; see also Bernardo, 1996; Mousseau and Fox, 1998; Wade, 1998; Marshall and Uller, 2007 for reviews). In contrast, the small compact size and the highly reduced cytoplasm of the animal male gamete – the sperm – was one of the main reasons for the assumption that paternal condition plays little to no role in determining offspring phenotype. This assumption has been overturned just over decade ago and it is now recognised that sperm contribute more than the paternal haploid genome (Krawetz, 2005). In this review, I provide an overview of the potential non-genetic mechanisms and factors transferred via sperm into the zygote. I discuss the evidence for their effects across generations, their putative causes and potential consequences in an evolutionary context. This is by no means a complete account and only provides small insights into a highly complex and fascinating world, but it may stimulate further research into the many processes that can be summarized as “sperm factor”.

## 60 **Male condition and sperm phenotype**

Male condition is affected by environmental factors such as diet, temperature and social interactions and these effects are often reflected in the characteristics of a male's ejaculate. Nutritional stress is known to negatively affect sperm quality and can lead to an increase in the number of

65 malfunctioning and morphologically abnormal sperm, which in turn may affect male fertilisation success (Gage and Cook, 1994; Merrells *et al.*, 2009; Perry and Rowe, 2010; Tigreros, 2013; Kahrl and Cox, 2015;). Similarly, variation in environmental temperature affects ejaculate traits such as sperm number and sperm morphology in ectotherm insects (Fox *et al.*, 2006) and fish (Breckels and Neff, 2013) but also in endotherm mammals (e.g. Al-Khanaan *et al.*, 2015). Finally, aspects of male social environment such as male:female ratio and the perceived intensity of sperm competition are known to affect sperm numbers (Arnaud *et al.*, 2001; Pilastro *et al.*, 2002; Pizzari *et al.*, 2003), sperm swimming velocity (Burness *et al.*, 2004) and sperm morphology (Crean and

75 Marshall, 2008; Immler *et al.*, 2010). However, while these environmentally induced changes in ejaculate traits are well established, the potential consequences of such changes for the next generation are poorly understood. In order to estimate the importance of paternal effects we need to understand the non-genetic factors carried by sperm and which part of the zygotic

80 development they might affect.

### **Why do paternal effects exist?**

While the evidence for an effect of the paternal condition on the offspring is rapidly mounting (e.g. Curley *et al.*, 2011; Soubry, 2015; Illum *et al.*, 2018 for

85 review), the evolutionary reason for the existence of paternal effects is less clear. Here below, I discuss four non-mutually exclusive hypotheses that may serve as possible explanations for the transfer of non-genetic information from the father to the offspring.

90 *Paternal effects are non-adaptive*

The transfer of non-genetic factors through sperm could be non-adaptive noise caused by physiological processes affecting the epigenetic mechanisms in the male germline in response to changing environmental conditions experienced by the father. Many of the experimental manipulations used to  
95 study paternal effects involve a change in the stress level experienced by the male for a defined period during life. Stress generally evokes strong physiological responses, which may negatively affect the germline and with that male reproduction (McGrady, 2009). These negative effects may include an increase in the production of reactive oxygen species (Dickinson and  
100 Chang, 2011) and elevated activity of repetitive elements (Capy *et al.*, 2000), both of which jeopardise the integrity of the genome and may increase mutation rates (Maklakov and Immler, 2016). Defense mechanisms of the genome against such mutagenic factors include DNA methylation, chromatin modifications and the production of small RNAs (sRNAs) including Piwi  
105 interacting (piRNAs) and microRNAs (miRNAs; Bartel, 2004; Klattenhof and Theurkauf, 2008; Siomi *et al.*, 2011; Ernst *et al.*, 2017). All three factors are known to be involved in mediating the possible effects of selfish genetic elements at the translational and post-translational levels. As a result, relevant epigenetic marks produced in protection of the germline genome may end up

110 in the mature gametes as relicts by chance rather than for adaptive reasons.  
At this stage, the non-adaptive hypothesis needs careful testing before we  
can exclude it with certainty.

*Paternal effects as an adaptive response to increase offspring fitness*

115 The transfer of information about the environmental conditions encountered  
by the parents to their offspring may be beneficial and provide an adaptive  
advantage to the offspring (Bonduriansky and Day, 2009; Turner, 2009). A  
mechanism that allows for such a transfer of information without modifying the  
genome may offer a flexible solution particularly in rapidly changing  
120 environments. A recent theoretical study described a positive feedback  
process where the parental phenotype favoured by environmental conditions  
gets progressively reinforced in the following generations through a learning  
mechanism (Xue and Leibler, 2016). Empirical evidence for such dynamics  
have been reported in *C. elegans* where small RNAs have been shown to be  
125 inherited for several generations without further additional stimulation with the  
help of RNA-dependent RNA polymerases (Rechavi *et al.*, 2011; Ashe *et al.*,  
2012; Gu *et al.*, 2012; Rechavi *et al.*, 2014). A recent study in *C. elegans*  
provided direct evidence for such a feedback loop determining the duration of  
transgenerational inheritance of small RNAs (Houri-Ze'evi *et al.*, 2016).  
130 Similarly, the ability of prions to assume a self-templating fold mechanism  
(Harvey *et al.*, 2018) suggests that these have the potential to maintain  
themselves in a self-regulating manner over many generations. Such  
genome-independent systems could be a way to memorise past conditions  
and transfer relevant information across generations for swift adjustments to

135 slow or rapid environmental changes despite the rigidity of the underlying  
genome.

*Paternal effects to mediate sexual conflict*

The inheritance of a paternal and a maternal genome creates a conflict  
140 between males and females over allele expression at heterozygous loci in the  
offspring (Arnqvist and Rowe, 2005). Epigenetic factors may further contribute  
to this conflict if they are inherited at an equal rate from both parents, but they  
may also offer a mechanism to resolve the conflict. Genomic imprinting is an  
epigenetic mechanism, which determines expression of an allele according to  
145 its parental origin (Reik and Walter, 2001). The three main theories proposed  
for the evolution of genomic imprinting are the kinship theory (Haig, 2000), the  
sexual antagonism theory (Day and Bonduriansky, 2004; Bonduriansky, 2007)  
and the maternal-offspring co-adaptation theory (Wolf and Hager, 2006; Wolf  
and Hager, 2009, all reviewed in Patten *et al.*, 2014). The question at the  
150 heart of all three theories is the conflict between the parents over gene  
expression in their offspring at heterozygous loci. The aspect that varies  
between the theories is the nature of the involved parties (parent-offspring,  
male-female or all of them together etc.) and of the resolving mechanism.

155 Similar to the hypothesis presented for sexual conflict, other genetic conflicts  
have been proposed as a possible explanation for sperm carrying RNAs  
(Holman and Price, 2014; Hosken and Hodgson, 2014). These authors  
suggested that RNAs mediate potential genomic conflicts not only between  
males and females but also between the diploid male and its haploid sperm,

160 and among the different sperm within an ejaculate. Given the sheer variation  
of RNAs present within each sperm they possibly cover several of these  
functions.

*Paternal effects to control selfish genetic elements*

165 The genomic conflict arising between the genome and selfish genetic  
elements may provide another explanation for the evolution of  
transgenerational epigenetic mechanisms (Holman and Price, 2014). The  
transfer of defense mechanisms against the detrimental effects of stressful  
environments from the male germline to the zygote would allow the protection  
170 of the zygotic genome during the sensitive stages of early development. The  
findings of variation in small RNA profiles, methylation patterns and chromatin  
structure in response to environmental stressors in sperm and the resulting  
offspring appear to be in line with this idea. However, we still know relatively  
little about the association between transposable elements (TEs) and  
175 epigenetic marks and mechanisms. A recent study in *Arabidopsis thaliana*  
showed that changes in methylation patterns and increased levels of gene  
expression were directly associated with *de novo* insertions of TEs in the  
immediate vicinity of affected genes (Stuart *et al.*, 2016). Whether similar  
associations exist in the male germline and/or in the zygote is currently not  
180 known.

Epigenetic factors and RNAs in the sperm may also derive from segregation  
distorting alleles that involve the incapacitation/killing of sperm or zygotes  
carrying alternative alleles (Holman and Price, 2014). This suggestion is



185 purely hypothetical and needs careful testing. But if such a mechanism exists,  
it would potentially affect male fertility. An association between male fertility  
and certain RNAs has been shown in humans but the mechanisms involved  
are unknown (Jodar *et al.*, 2012).

190 The hypotheses outlined above for the evolution of genetic imprinting and the  
role of RNAs as signals among different units may apply to any epigenetic  
factor transmitted via sperm. Males can undoubtedly benefit from transmitting  
more than just a genome in their gametes, and the idea that these  
mechanisms are adaptive is enticing. Testing the non-adaptive alternative is  
195 therefore even more important and necessary. It will be exciting to examine  
the different hypotheses and understand more about the evolutionary  
dynamics involved. This should be increasingly possible with the steadily  
improving methods available in genomics, transcriptomics and proteomics.

## 200 **Which non-genetic components does a sperm transfer to the zygote?**

Beside the nuclear genome, sperm are known to contain a range of  
epigenetic elements, which are transferred into the zygote upon fertilisation,  
including chromatin modifications, RNAs and proteins (reviewed in Dadoune,  
2009; Carrell, 2012; Casas and Vavouri, 2014; Rando, 2016; Figure 1). Here  
205 below, I provide a brief overview of the currently known factors and present  
examples for the ways these factors might affect processes in the zygote and  
beyond. I am using the term “epigenetics” in a broad sense and follow  
Henikoff and Greally’s (2016) definition, where any cellular memory not  
encoded in the genetic code is included. Genome-carrying cell organelles

210 such as mitochondria are therefore not included even though these may be  
inherited through sperm in rare occasions in some organisms, such as insects  
(Wolff *et al.*, 2012), mammals (Zhao *et al.*, 2004) and birds (Alexander *et al.*,  
2015) and regularly in others such as mussels (Sutherland *et al.*, 1998;  
Zouros, 2000). Even with this relatively restricted definition of the term  
215 epigenetic, condition dependent transgenerational effects may be harder to  
identify than assumed, and some of the aspects that may need further  
investigation are described in the section *Current challenges and future  
directions* below.

## 220 *DNA methylation/acetylation*

DNA methylation is probably the most studied epigenetic mark and is  
assumed to play a major role in the transfer of non-genetic information across  
generations. DNA methylation in combination with histone modifications (see  
section below) plays a key role in regulating gene expression in the germ cells  
225 and thereby contributes to three key processes: (I) the specification and  
formation of primordial germ cells, (II) the genome-wide erasure and re-  
establishment of germline-specific patterns in the embryo and sex-specific  
patterns during gametogenesis and (III) the establishment of sex-specific  
patterns typical for mature male and female gametes (reviewed in Allegrucci  
230 *et al.*, 2005). Given their key role in governing gene expression throughout  
development, it is not surprising that paternal condition affects methylation  
patterns in the offspring. Fathers kept on a high fat diet in Sprague-Dawley  
rats for example sired daughters with impaired insulin secretion and glucose  
tolerance. Their female offspring exhibited altered expression in 642

235 pancreatic islet genes with some of the key genes being hypomethylated (Ng  
*et al.*, 2010). More generally, environmental changes during early  
developmental stages seem to have a major impact on germline methylation  
patterns (see Faulk and Dolinoy, 2011 for review).

240 The molecular mechanism is based on the binding of a methyl/acetyl group to  
a DNA molecule, which may affect the transcriptional activity of the underlying  
gene without changing the genetic code. The percentage of methylation  
inherited from the father through sperm varies markedly across taxa and may  
range from fully maternally inherited to largely paternally inherited patterns. In  
245 house mice *Mus musculus* (and other mammals), the methylation structure in  
the developing zygote is re-structured during early embryogenesis following  
the maternal template and paternal marks are mostly removed (see Daxinger  
and Whitelaw, 2012 for review). In contrast, in zebrafish *Danio rerio*, the  
paternal methylation pattern forms the template and the maternal methylation  
250 pattern is largely restructured according to the information coming from the  
father (Potok *et al.*, 2013; Jiang *et al.*, 2013). These taxonomic differences in  
methylation inheritance are currently not explained and possible evolutionary  
reasons need to be tested.

#### 255 *RNA families*

Sperm contain many families of RNAs, which may be transferred into the  
zygote during fertilisation and may therefore affect processes involved during  
early embryogenesis (Dadoune, 2009). These RNA families include  
messenger RNAs (mRNA; Alcivar *et al.*, 1989; Ostermeier *et al.*, 2002; Yang

260 *et al.*, 2009; Bonache *et al.*, 2012), micro RNAs (miRNAs; e.g. Krawetz *et al.*,  
2011), Piwi interacting RNAs (piRNAs; e.g. Krawetz *et al.*, 2011), transfer  
RNA derived small RNAs (tRNAs; e.g. Peng *et al.*, 2012) and a number of  
other to date un-specified RNA families. mRNAs are a large group of different  
molecules that are the direct result of gene transcription and are therefore  
265 also known as “coding” RNAs. The mRNA content in sperm is relatively low  
compared to any other cell type, and their origin (i.e. pre- versus postmeiotic)  
and role need further investigation.

The three remaining families (i.e. miRNAs, piRNAs and tRNAs) belong to the  
270 group of “small non-coding” RNAs (sRNAs) as they are transcribed from non-  
coding regions of the genome, and for many, their origin and function is still  
unknown. miRNAs are short (about 22-nucleotides) molecules that are  
involved in RNA silencing and regulation of gene expression at the  
transcriptional and post-transcriptional stages (Bartel, 2004). They may  
275 mediate the activity of selfish genetic elements by triggering small interfering  
RNAs (siRNAs) in a highly specialised and pathway specific manner (Creasey  
*et al.*, 2014). Similarly, piRNAs (21-32 nucleotides) in the germline are  
involved in the silencing of selfish DNA elements and the maintenance of  
DNA integrity through the formation of RNA-protein complexes that act at the  
280 transcriptional and post-transcriptional levels (Klattenhof and Theurkauf,  
2008; Siomi *et al.*, 2011; Ernst *et al.*, 2017). However, the exact mechanisms  
and origins of piRNAs are currently elusive. tRNAs (sometimes also referred  
to as tsRNAs) may vary in length (from 20 nucleotides into the range of  
piRNAs) and have been assumed to be the result of transmitter RNA

285 degradation until they were clearly identified as a distinct group of small non-  
coding RNAs (Lee *et al.*, 2009). Observations in house mice *M. musculus*  
suggested that in testicular sperm, the tRNA content is low but increases with  
maturation through the fusion with epidyosomes (Sharma *et al.*, 2016). The  
same study also reported that the function of these tRNAs is to repress genes  
290 associated with the selfish element MERVL active in preimplantation  
embryos.

The total amount of RNA molecules transferred through sperm is vanishingly  
small compared to the RNAs present in the egg. Nevertheless, several  
295 families of RNAs have been reported to be involved in non-genetic inheritance  
of paternal conditions across generations. miRNAs and piRNAs were  
differentially expressed in the sperm of male house mice *M. musculus*  
exposed to traumatic stress during the juvenile life stage compared to sperm  
of control male mice (Gapp 2014). The injection of these differentially  
300 expressed RNAs into early zygotes lead to similar offspring phenotypes as  
those observed in the experiments using traumatised males as fathers.  
Furthermore, miRNAs were involved in the transmission of chronic stress  
responses experimentally evoked in adult male mice to their offspring  
(Rodgers *et al.*, 2013). The precise role of tRNAs needs further investigation  
305 but they seem to affect gene expression during early embryo development  
(Sharma *et al.*, 2016).

### *Proteins*

Sperm are composed of a wide range of proteins located on the sperm  
310 surface, in the acrosome (where present), in and around the nucleus and  
even in the flagellum. The sperm proteome as a whole has been analysed  
with respect to human infertility and 20 proteins have been identified to be  
associated with fertility issues (Lefievre *et al.*, 2003; Pixton *et al.*, 2004; Rawe  
*et al.*, 2008). A similar study in the house mouse *M. musculus* shortlisted 132  
315 proteins that may affect fertility, some of which seem to be evolutionarily  
preserved across taxonomic groups (Chu *et al.*, 2006). These findings  
suggest a potential major role for proteins in transgenerational epigenetics.

In fact, in non-rodent mammalian fertilisation, the centriole-centrosome is  
320 inherited through the sperm and acts as a template for all subsequent cell  
divisions from early embryogenesis into adulthood. Any malformations of this  
complex result in severe infertility due to disruption or insufficiency during  
mitotic divisions and may hence cause developmental problems anywhere  
from interrupting the first mitotic divisions to causing embryonic malformations  
325 (Schatten and Sun, 2013). The centriole-centrosome complex likely varies in  
its shape and therefore function also among fertile males, and these more  
subtle variations may contribute to the fitness and performance of the  
offspring in the next generation.

330 In a recent review, Harvey *et al.* (2018) proposed that prions are ideal  
candidates for non-genetic transgenerational inheritance due to their  
conformational flexibility and their ability to transform into self-templating folds,  
which allows them to proliferate independently even across generations.

Prions are considerably more stable during meiotic processes compared to  
335 other epigenetic factors experiencing major re-structuring (Cox, 1965; Young  
and Cox, 1971). The independence and stability of prions may imply that  
protein-based transgenerational inheritance could be important but the idea  
needs careful testing.

#### 340 *Histone modifications*

Although histone modifications could be regarded as part of the sperm  
proteome, I discuss them separately as they have received a lot of attention in  
the context of trans-generational epigenetics. Modifications of the histones are  
assumed to affect gene expression and therefore may play a key role in gene  
345 regulation (e.g. Kouzarides, 2007). Gene regulation is particularly important  
during the early stages of development and any marks inherited from the  
father may contribute to embryonic gene expression – with potential effects  
later on in life. In mammalian sperm, 90 (in humans) to 95% (in house mice)  
of histones are replaced by protamines during spermatogenesis, and the  
350 remaining histones may undergo post-translational modifications affecting  
gene expression at these loci (Luense *et al.*, 2016). These post-translational  
modifications may regulate gene expression during spermatogenesis and  
during early embryo development (Brykczynska *et al.*, 2010; Hammoud *et al.*,  
2011; Erkek *et al.*, 2013; Brunner *et al.*, 2014). In human sperm, histone  
355 modifications appear to be particularly enriched around developmental loci.  
Dimethylated lysine 4 on histone H3 (H3K4me2) for example, is found at  
promoter loci, whereas H3K4me3 is found in large clusters of paternally  
expressed imprinted genes, miRNAs and HOX genes (Hammoud *et al.*,

2009). These patterns also seem to hold for the zebrafish *Danio rerio*, where  
360 sperm retain the histones and lack protamines altogether, but chromatin  
markers such as permissive H3K4me3 with or without repressive H3K9me3 or  
H3K27me3 are associated with developmental loci (Lindeman *et al.*, 2011). A  
study manipulating the dietary conditions in male house mice observed  
differential gene expression in the next generation and found a consistent  
365 decrease in H3K27me3 at the promoter of monoamine oxidase in sperm of  
low-protein diet males compared to control males (Carone *et al.*, 2010).

### **Current challenges and future directions**

The study of paternal epigenetic effects inherited across generations is still in  
370 its early days and many fundamental questions are currently unanswered.

The many unfilled gaps and fundamental unknowns put limitations to our  
ability to summarise the relative importance, prevalence, and/or impact of  
each of the factors discussed. It may be worth identifying some of the key  
aspects that we should focus on in the near future.

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The term “sperm factor” may be somewhat misleading in being an  
oversimplification of what is clearly a varied set of highly complex factors. One  
of the questions is therefore: How are the different mechanisms linked?

Understanding whether the different epigenetic components act

380 independently, complementarily, additively, or interactively and how these  
interactions and the resulting effects may be context-dependent are some of  
the challenges we are currently facing. The interaction between some of the  
factors such as the tight linkage between DNA methylation and histone



modifications for the regulation of gene expression during proliferation and  
385 differentiation of the germline is relatively well understood. In contrast, other  
factors are still largely a black box (piRNAs), and many have not even been  
properly identified yet (other small RNAs). Carefully designed experiments  
combined with the latest –omics technology may be a valuable way to gain  
insights into what are clearly highly complex processes.

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Another currently open question is whether the non-genetic transfer of  
information in sperm is truly “non-genetic” or whether there is a causal  
connection between the non-genetic information and the underlying genome.  
Non-genetic factors may fall into one of three possible categories: (I)  
395 independent of sequence variation, (II) partially dependent on sequence  
variation, and (III) completely dependent on sequence variation (based on  
epiallelic variation as proposed by Richards, 2006). An additional aspect that  
needs to be considered is whether the transfer of information is based (A)  
purely on transmitted genes or (B) on a combination of transmitted genes and  
400 non-genetic material. In case III, all the observed variation should be  
explained by focusing exclusively on sequence variation and the distinction  
between scenario A and B is not necessary. However, in cases I and II,  
sequence variation will not explain everything as non-genetic material may be  
generated independently and add variation through non-genetic mutations  
405 occurring between transcription events. Performing experimental  
manipulations of paternally experienced environmental conditions in  
combination with long-read DNA sequencing, RNA sequencing of different

RNA families, ChIP sequencing and bisulfite sequencing is not an easy but a promising way forward to answer these questions.

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The importance of the relative timing and duration of changes in environmental conditions experienced by a male to affect the following generation(s) is still poorly understood. In mammals (and probably most other taxa), early embryo development is a particularly sensitive period and methylation patterns and histone modifications are strongly affected by environmental conditions during this time (reviewed in Faulk and Dolinoy, 2011). However, effects across generations have also been shown in studies where males were exposed to stressful environments as juveniles before sexual maturity (e.g. Gapp *et al.*, 2014), during adulthood (e.g. Carone *et al.*, 2010) or both (e.g. Rodgers *et al.*, 2013). It would be interesting to understand, which epigenetic factors are mostly affected by environmental conditions in the male germline during each of these life stages and how strong the observed transgenerational effects are relative to each other.

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Of particular relevance for the fields of ecology and evolution is the question about the stability of epigenetic alterations. While some epigenetic marks are stable and conserved even across taxa (Provataris *et al.*, 2018 ), others are seemingly more apt to change. Having said that, even sRNAs can be transferred across many generations without further stimulation in a self-regulating process (Rechavi *et al.*, 2014) suggesting that such systems may provide a reliable way to memorise environmental conditions. Understanding

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the flexibility and stability of epigenetic mechanisms is important to fully assess their relative contribution to inheritance.

435 Finally, ejaculates generally consist of more than just sperm, and we know that the content of seminal fluids may have severe effects on female fitness (Chapman *et al.*, 1995; Wolfner, 2002), and also on their offspring (Chapman *et al.*, 2001; Crean *et al.*, 2014; Crean *et al.*, 2016). Controlling for such effects and disentangling factors carried by sperm from factors in the seminal  
440 fluid will be imperative when studying the various mechanisms.

In summary, non-genetic factors transferred through the sperm into the zygote are very likely to affect the resulting generation(s) and this in itself is a very important insight. We now need to understand, which mechanisms contribute  
445 to this transfer of information and how and what the true purpose of non-genetic information transferred in sperm across generations is. With a great range of novel tools becoming available and increasingly affordable we should be able to address these important questions.

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## Conflict of interest

The authors declare no conflict of interest.

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**Figure 1:** Illustration of non-genetic components transferred via sperm from the father to the offspring and their putative effects in the offspring. The description of the effects is very general as many of them are currently still poorly understood.

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Figure 1

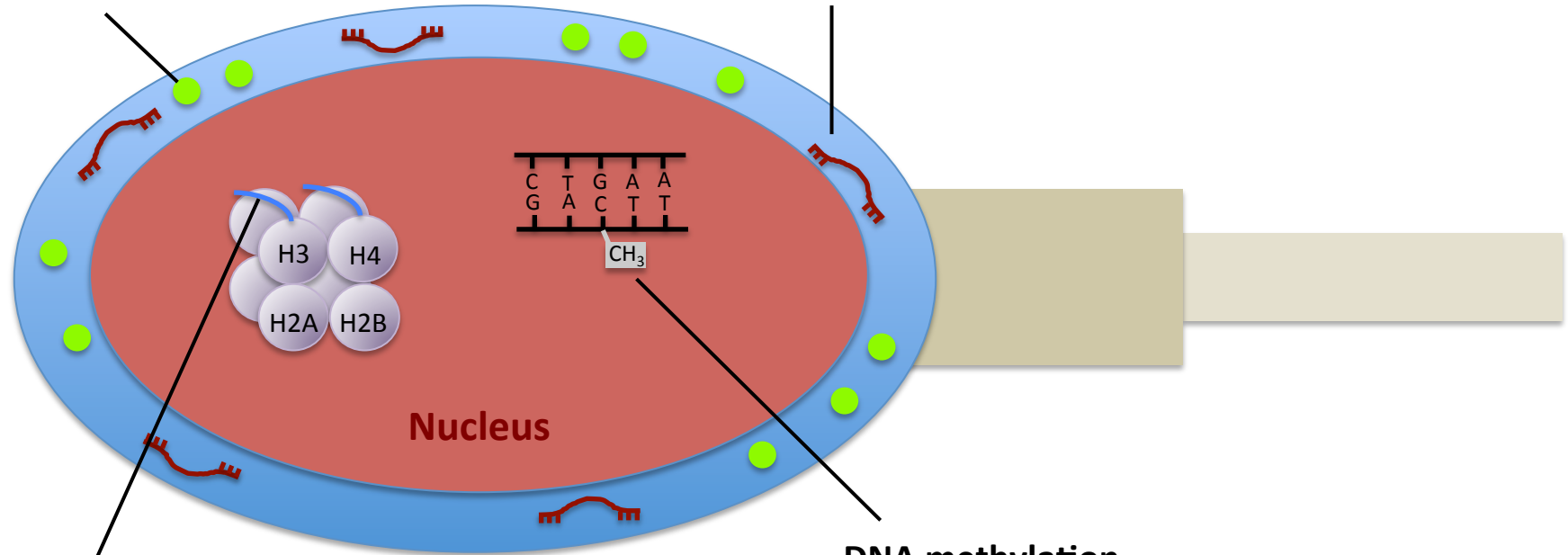
## Sperm factors

### Proteins

Cell divisions, other effects?

### RNAs

Gene regulation,  
TE activity



### Histone modifications

Gene regulation, TE activity

### DNA methylation

Gene regulation, TE activity